31st 2000 INRC

Conference Program

Plenary lectures, symposia, and oral presentations will be held in Kane Hall on the University of Washington campus. Posters should be mounted in the Grand Ballroom no later than 12:45 pm on Sunday (July 16th) and remain up until 15:30 Wednesday July 18th..

Saturday, July 15, 2000

13:00 – 19:30	Registration, Kane Hall Lobby
19:30 – 21:30	Reception, Walker Ames Room, 2nd floor, Kane Hall

Sunday, July 16, 2000

07:00 – 08:30	Registration, Kane Hall Lobby	
07:00 – 08:30	Continental Breakfast, Walker Ames Room, Kane Hall	
08:30	Welcome	Charles Chavkin
08:35	**Plenary Lecture**	*"Beta arrestins: Traffic cops of cell signalling"*
	Robert J. Lefkowitz	(Introduced by Lakshmi Devi)
09:35 – 12:30	**Symposium 1:**	*"Drug Dependence Mechanisms: Compensatory Responses to Chronic Opioids,"*

Chairs: MacDonald Christie and Charles Chavkin

09:35 *Factors controlling drug-seeking behaviour following withdrawal from heroin and cocaine self-administration,* T. J. De Vries, L. J. M. J. Vanderschuren, J. R. Homberg, E. H. Jacobs, A. B. Smit and A. N. M. Schoffelmeer

10:00 *Chronic exposure to morphine initiates new opioid receptor-coupled signaling strategies,* A. R. Gintzler and S. Chakrabarti

10:25 *Differences in gene expression in the nucleus accumbens following opiates,* J. Eberwine

10:50 - 11:20 Coffee Break

11:20 *Functions of the mesocorticolimbic circuit at the millennium,* S. J. Henriksen

11:45 *Hippocampal LTP is suppressed and becomes drug-dependent after chronic exposure to opiates,* G. Bao, L. Pu, N. Xu, L. Ma and G. Pei

12:00 *Multiple cellular mechanisms of opioid withdrawal,* C. W. Vaughan, E. E. Bagley, M. Connor and M. J. Christie

12:15 *Chronic morphine treatment alters nucleus accumbens NMDA receptor properties,* G. Martin, S. Ahmed, G. F. Koob, L. deLecea and G. R.Siggins

12:30 *Morning session ends*

12:45 – 13:30 Buffet Lunch, Grand Ballroom, 2nd floor Husky Union Building (HUB)

13:30 – 15:30 Poster Session 1, Grand Ballroom, 2nd floor Husky Union Building (HUB)

15:45 *Return to Kane Hall*

16:00 – 19:10 Symposium 2: *"Opiates and the Genome"*

Chairs: George Uhl and Volker Höllt

16:00 *Introduction: "Upstream" and "Downstream" genes,* G. Uhl

16:10 *Genes regulated by μ agonists and μOR deletion: microarray studies,* Q. R. Liu, I. Sora, S. Hall and G. Uhl

16:30 *Transcriptional regulation and genetic variations of opioid receptor genes,* V. Höllt, T. Koch, J. Kraus, T. Kroslak, P. Mayer, S. Schulz and A. Zimprich

16:55 - 17:20 Coffee Break, Walker Ames Room, Kane Hall

17:20 *Regulation of mu opioid receptor genes,* H. H. Loh

17:45 *A mouse model of substance abuse,* D. E. Grice, T. N. Ferraro, G.T. Golden, R. J. Buono and W.H. Berrettini

18:10 *Use of cDNA microarrays to analyze gene expression response to opiates,* S. Nelson, D. Ding, E. Castro and C. Evans

18:30 *Single-nucleotide polymorphisms in the human mu opioid receptor gene alter receptor-CaM binding and G protein coupling*, D. Wang, J. M. Quillan and W. Sadée

18:45 *Gene expression profiles in acute morphine dependence and withdrawal*, S. R. Nagalla, S. Baggia, P. Pattee and J. K. Belknap

19:00 *Discussion*, M. Iadarola

19:10 Concierge Dinners

Monday, July 17, 2000

07:00- 08:30 Registration, Kane Hall Lobby

07:00 – 08:30 Continental Breakfast, Walker Ames Room, Kane Hall

08:30 **Plenary Lecture II** *"Molecular substrates of opiate addiction"*

Eric J. Nestler (Introduced by Charles Chavkin)

09:30 – 12:30 **Symposium 3:** *"Learning Mechanisms Controlled by Opioids: Plasticity Underlying Tolerance and Addiction"*

Chairs: John Williams and George Siggins

9:30 *The contribution of the anti-opioid peptide cholecystokin (CCK) to morphine analgesia and tolerance*, J. Mitchell

10:00 *Synaptic plasticity in the prefrontal cortex and the nucleus accumbens: implications for reward directed behaviors*, A. B. Mulder, R. E. Nordquist, O. B. Örgüt and C. M. A. Pennartz

10:25 *Opiate-induced synaptic plasticity in hippocampus*, C. Chavkin

10:50 - 11:20 Coffee Break, Walker Ames Room, Kane Hall

11:20 *Modulation of LTP and LTD in VTA and nucleus accumbens in vitro by amphetamine*, J. Kauer

11:45 *Chronic morphine induces synapse-specific changes in the nucleus accumbens*, J. M. Brundege and J. T. Williams

12:00 *Targeting of mu-opioid receptors in ventral tegmental area neurons that project to medial prefrontal cortex*, A. L. Svingos, M. Garzón, E. E. O. Colago and V. M. Pickel

12:15 *Conditioned place preference (CPP) as a model for relapse of drug use and the effect of peripheral electrical stimulation (PES),* J. S. Han, B. Wang and F. Luo

12:30 *Morning session ends*

12:45 – 13:30 Buffet Lunch, Grand Ballroom, 2nd floor Husky Union Building (HUB)

13:30 – 15:30 Poster Session 2, Grand Ballroom, 2nd floor Husky Union Building (HUB)

15:45 *Return to Kane Hall*

16:00 – 19:00 Symposium 4: *"Role of Pain in Regulation of Opiate Tolerance"*

Chairs: Tsutomu Suzuki and Howard Fields

16:00 *Introduction,* T. Suzuki

16:05 *Enhanced spinal nociceptin system is involved in the development of morphine tolerance and dependence,* H. Ueda

16:30 *Chronic morphine up-regulates cell surface delta opioid receptors: implications for pain control,* A. Beaudet, A. Morinville, M. C. Lee and C. M. Cahill

16:55 - 17:15 Coffee Break, Walker Ames Room, Kane Hall

17:15 *Identification of a functionally important nociceptive pathway that is not vulnerable to opioids,* R. Elde

17:40 *Modulation of rewarding effects of opiates by nociceptive stimuli,* T. Suzuki, Y. Kishimoto and M. Narita

18:05 *Descending facilitation in opioid-induced pain and antinociceptive tolerance,* T. W. Vanderah, M. H. Ossipov, T. P. Malan, Jr., J. Lai and F. Porreca

18:30 *Antinociceptive responses induced by opioid compounds in two different lines of MOR knockout mice.* R. Maldonado, M. A. King, J. M. Mitchell, O. Pol, A. G. P. Schuller, H. Matthes, G. W. Pasternak, B. L. Kieffer and J. E. Pintar

18:45 *Prodynorphin "knock-out" mice do not show sustained neuropathic pain or spinal opioid tolerance,* J. Lai, T. P. Malan, Jr., Z. Wang, L. Gardell and F. Porreca

19:00 Concierge Dinners

Tuesday, July 18, 2000

07:00 – 08:30	Registration, Kane Hall Lobby	
07:00 – 08:30	Continental Breakfast, Walker Ames Room, Kane Hall	
08:30	**Plenary Lecture III**	*"Signaling pathways in cells and retro-viruses investigated by structural biology and chemical approaches"*
	Bernard P. Roques	(Introduced by Eric Simon)
09:30 – 12:30	**Symposium 5:**	*"Regulation of Opioid Receptor Signalling"*
	Chair: Horace Loh	

9:30 *Introduction* H. H. Loh

9:35 *Consequences of opioid receptor phosphorylation*, O. Maestri-El-Kouhen, H. H. Loh and P.-Y. Law

10:00 *Mechanisms of agonist-induced down-regulation of the human opioid receptor*, J.-G. Li, J. L. Benovic and L.-Y. Liu-Chen

10:25 *Relationship of ligand-induced ERK activation and mu opioid receptor phosphorylation*, J. B. Wang, W. Guang and P. Shapiro

10:50 - 11:20 Coffee Break, Walker Ames Room, Kane Hall

11:20 *Significance of excitatory G_s-coupled opioid receptor functions in vivo*, S. M. Crain

11:45 *Regulation of opioid receptor signaling by receptor dimerization*, J. L. Whistler and M. von Zastrow

12:00 *Molecular mechanism of mu opioid receptor desensitization.* J. Celver, A. Kovoor, J. Lowe, V. V Gurevich and C. Chavkin

12:15 *Calcium/calmodulin binding to mu receptors and its role in opioid activation of ERK*, M. Belcheva, M. Szûcs, D. Wang, W. Sadée and C. Coscia

12:30 *Morning session ends*

12:45 - 16:00 Excursions/ free afternoon: Self-guided walking tour of Seattle Market and Waterfront Three 47-passenger buses will cycle between the Conference Center and Pike Place Market between 12:45 - 16:30

19:00-21:20 **Symposium 6: Evening Oral Session** *"Late Breaking News"*

19:00 *Opioid receptor nomenclature*, B.M. Cox

19:30 *δ- and μ-opioid receptor-deficient mice exhibit opposing alterations of emotional responses*, B. L. Kieffer, D. Filliol, S. Ghozland, J. Chluba, M. Martin, H. Matthes, F. Simonin, K. Befort, C. Gavériaux-Ruff, A. Dierich, M. LeMeur, O. Valverde and R. Maldonado

19:55 *Amphetamine treatment and withdrawal alters limbic system network properties*, A. A. Grace, S.-P. Onn and H. Moore

20:20 *A membrane sorting mechanism that mediates downregulation of opioid receptors after endocytosis by clathrin-coated pits*, M. von Zastrow, P. Tsao, J. Whistler, H. Deacon and T. Cao

20:35 *A collaborative report on the development of DALDA and [DMT^{1}]DALDA*, H. H. Szeto, P. W. Schiller, M. Shimoyama, N. M. Lee and D. M. Desiderio

20:50 *Regulation of adenylyl cyclase isozymes by opiates: insight on the mechanism of adenylyl cyclase superactivation*, Z. Vogel, K. Eckhardt, D. Steiner, E. Gershon, M. Bayewitch, I. Nevo and O. Zagoory

21:05 *Differential expression of eight splicing variants are directed by a new promoter of the mouse mu opioid receptor gene (MOR-1)*, Y.-X. Pan, J Xu, G. Rossi, M.-M. Xu, L. Mahurter, E. Bolan and G. W. Pasternak

21:20 *Evening session ends*

Wednesday, July 19, 2000

07:00 – 08:30 Registration, Kane Hall Lobby

07:00 – 08:30 Continental Breakfast, Walker Ames Room, Kane Hall

08:30 **INRC Founders' Lecture:** *"1965 - 1975: A renaissance decade in opiate research"*

Brian M. Cox (Introduced by Charles Chavkin)

09:30 – 12:40 **Symposium 7:** *"Novel Approaches to the Treatment of Drug Abuse and Pain"*

Chairs: Jean Bidlack and Phil Portoghese

09:30 *Introduction* J. M. Bidlack

9:35 *NIDA medications efforts in developing treatments for opiate and stimulant dependence*, F. Vocci

10:00 *Evaluation of opioids for the treatment of cocaine abuse,* S. S. Negus and N. K. Mello

10:25 *Disconnecting pain from the brain with ligand targeted cytotoxins,* M. Iadarola

10:50 - 11:20 Coffee Break, Walker Ames Room, Kane Hall

11:20 *How does nociceptin bind and activate the ORL1 receptor?* J.-Cl. Meunier

11:45 *Pharmacological characterization of J-113397, a potent ORL1 receptor antagonist,* S. Ozaki, S. Okuda, M. Miyaji, T. Tanaka, H. Kawamoto, Y. Itoh, Y. Iwasawa and H. Ohta

12:10 *Regulatory mechanisms of proorphanin FQ and proenkephalin gene expression in neuronal and glial cells,* B. Buzas, J. Rosenberger and B. M. Cox

12:25 *A novel peptide expressed in the mammalian brain produces hyperalgesia and nociceptive behavior in rats,* L. Negri, R. Lattanzi, E. Giannini and D. Melchiorri

12:40 *Morning session ends*

12:45 – 13:30 Buffet Lunch, Grand Ballroom, 2nd floor Husky Union Building (HUB)

13:30 – 15:30 Poster Session 3, Grand Ballroom, 2nd floor Husky Union Building (HUB)

15:30 **TAKE DOWN POSTERS**

15:30 An Update on Recent Developments at NIDA: NIDA's Cell Biology and Genetics Program, Jonathan Pollock. Grand Ballroom, 2nd floor Husky Union Building (HUB)

16:00-16:30 INRC Business Meeting

17:30 Conference Banquet
Buses will leave promptly at 5:30 pm from Hotels (University Inn, Meany Hotel, Silver Cloud Inn and Haggett Hall) to meet the Ferry boat to the Kiana Lodge
(return to hotels at ~22:30)

Thursday, July 20, 2000

08:00 – 09:00 Registration, Kane Hall Lobby

08:00 – 09:00 Continental Breakfast, Walker Ames Room, Kane Hall

09:00 – 11:35 Symposium 8 *"Opioid Receptor Structure: Dimers, Variants, Polymorphisms"*

Chairs: Lakshmi Devi and Susan George

9:00 *Introduction,* L. A. Devi

9:05 *Oligomerization of dopamine and serotonin receptors: insights into receptor structure and trafficking,* S. R. George, S. P. Lee and B. F. O'Dowd

9:30 *A role for dimerization in opioid receptor cross-talk,* L. A. Devi, B. Jordan, I. Gomes, N. Trapraidze, V. Nagy and R. Nivarthi

9:55 *Bivalent ligands as probes to evaluate opioid receptor dimer models,* P. S. Portoghese, G. Poda, D. J. Daniels and D. M. Ferguson

10:20 - 10:50 Coffee Break, Walker Ames Room, Kane Hall

10:50 *Genetic variability of the human mu opioid receptor gene and its potential functional significance,* M. R. Hoehe

11:05 *Assessment of signal transduction events in living cells using bioluminescence resonance energy transfer (BRET)* S. Angers, E. Joly, D. Chelsky and M. Bouvier

11:20 *Receptor domains involved in kappa-delta heterodimerization,* F. Meng, S. Evans, G. Bonner, M.-H. Kabbaj, L. Taylor and H. Akil

11:35 End of the Meeting

Poster Session 1: (Sunday, July 16, 13:30 – 15:30, Grand Ballroom)

Gene Expression, Genetics and Heredity

Sun01: *RNA profiling in a mouse model of substance abuse,* J. D. Buxbaum, N. S. M. Geoghagen, G. Smith, G. Golden, W. H. Berrettini and D. E. Grice

Sun02: *Recombinant HSV-1 mediated transfer of proenkephalin A gene into sensory neurons of arthritic rats - improved locomotion and reduced hyperalgesia,* F. Cesselin, J. Bras, C. Beaufour, M. Hamon and M. Pohl

Sun03: *Cross-sensitization between cocaine and morphine at the level of c-FOS expression,* M. Erdtmann-Vourliotis, P. Mayer, U. Riechert and V. Höllt

Sun04: *Single-stranded DNA binding complex involved in mouse μ-opioid receptor gene transcription,* J. L. Ko and H. H. Loh

Sun05: *Regulation of mu opioid receptor gene transcription by cytokines,* J. Kraus, C. Börner, K. Hickfang and V. Höllt

Sun06: *Human nociceptin/orphanin FQ receptor gene structure and polymorphism in heroin addiction,* K. S. LaForge, S. M. Leal and M. J. Kreek

Sun07: *Transcriptional regulation of mouse δ-opioid receptor gene,* H. C. Liu and H. H. Loh

Sun08: *MOR1 mRNA and electrophysiological responses to opioids in LC neurons,* G. T. Livezey, S. A. Schnell and M. W. Wessendorf

Sun09: *A delta opioid receptor lacking the 3rd cytoplasmic loop is generated in human malignomas,* P. Mayer, H. Tischmeyer, M. Jayasinghe, H. Teschemacher and V. Höllt

Sun10: *Quantitative trait loci (QTL) analysis of morphine-induced antinociception in the SMXA recombinant inbred strains of mice,* K. Mizuo, M. Narita, H. Ikeda, C. Inoue, M. Nishimura and T. Suzuki

Sun11: *Naloxone treatment of adult hippocampal progenitors,* P. A. I. Persson and P. S. Eriksson

Sun12: *Delta opioid receptor gene: transcriptional regulation,* D. Smirnov, H. J. Im and H. H. Loh

Sun13: *Mouse mu-opioid receptor distal promoter transcriptional regulation by SOX proteins,* X. Wu and H. H. Loh

Sun14: *Single nucleotide polymorphism of the human kappa opioid receptor,* V. Yuferov, K. S. LaForge, S. M. Leal and M. J. Kreek

Neuronal Circuits: Physiology and Anatomy

Sun15: *Immunohistochemical localization of a carboxy terminus epitope of the novel mu opioid receptor splice variant MOR-1C within the human spinal cord,* C. Abbadie, S. H. Gultekin and G. W. Pasternak

Sun16: *Dopaminergic neurotransmission in the nucleus accumbens of kappa-opioid receptor (KOR) knockout mice: an in vivo microdialysis study,* V. Chefer, T. Czyzyk, J. Pintar and T. S. Shippenberg

Sun17: *Coexpression of immunoreactivity for DOR1 and KOR1 in spinal dorsal horn,* J. Dooyema and M. Wessendorf

Sun18: *Lack of long-term potentiation in the dentate gyrus of μ opioid receptor-deficient mice.* H. Matthies, H. Schröder, A. Becker, H. Loh, V. Höllt, and M. Krug

Sun19: *Opioid and $GABA_A$ receptors are co-localized in rat brain,* A. Kalyuzhny, J. Dooyema and M. Wesendorf

Sun20: *Ethanol induction of FOS immunoreactivity in mu opiate receptor knockout mice,* C. M. Klodesky, Y. Jiang, H. H. Loh and S. L. Chang

Sun21: *Dynorphin B and endomorphin-1 inhibit the acetylcholine evoked currents in hair cells,* M. Lioudyno, G. Athas, M. Verbitsky, A. B. Elgoyhen, J. E. Zadina and P. S. Guth

Sun22: *Opiate induced mesolimbic dopamine release and locomotion in inbred mice,* N. P. Murphy, H. A. Lam and N. T. Maidment

Sun23: *Differential targeting of CB1 and μ-opioid receptors in the rat striatal patch,* J. J. Rodríguez, K. Mackie and V. M. Pickel

Sun24: *Frequent colocalization of the μ-opioid receptor and CaMKII in pain-processing brain regions,* S. Schulz, I. Brüggemann, D. Wiborny and V. Höllt

Sun25: *Cellular localization of opioid receptors in astrocytes and oligodendrocytes in newborn mouse brain,* A. Stiene-Martin, P. E. Knapp, K. Martin, J. A. Gurwell and K. F. Hauser

Sun26: *Sex differences in delta opioid receptor immunoreactivity in amygdala,* M. A. Wilson, F. Mascagni and A. J. McDonald

Pain and Inflammation

Sun27: *Expression of biologically active β-endorphin in K562 cells,* M. Bauer, W. Binder, S. König-Merediz, M. Schroff, B Wittig, M. Schäfer and C. Stein

Sun28: *Sex differences in kappa opioid antinociception,* S. Bernal and R. Craft

Sun29: *Clonidine-induced antinociception is reduced by dexamethasone in mice,* A. Capasso, M. Russo and A. Loizzo

Sun30: *Which types of opioid receptors are involved in the antinociceptive effects of RB101(S)?* G. Catheline, S. Le Guen, F. Noble, M.-C. Fournié-Zaluski, B. Roques, J.–M. Besson and J. Buritova

Sun31: *The MOR participates in DPDPE, DELT II-mediated spinal antinociception but is not required for DOR-α_2 adrenergic synergy,* X. H. Guo, C. A. Fairbanks, L. S. Stone and H. H. Loh

Sun32: *Very low dose naltrexone enhances morphine analgesia in humans,* B. Sherman, R. Barbier, S. Crain, M. Remien, F. Minn and D. Mehlisch

Sun33: *Endomorphin-2-like immunoreactivity in the mouse spinal cord decreases after chronic constriction injury,* R. R. Smith, S. Martin-Schild and J. E. Zadina

Sun34: *Anti-arthritic effects of the delta-selective opioid antagonist HS 378,* M. Spetea, J. Li, H. Schmidhammer, I. Bileviciute-Ljungar, T. Ahmad, M. Ahmed and A. Kreicbergs

Sun35: *The effect of noise stress-induced pain threshold on central monoaminergic neurones,* H. Y. Tsai, H. M. Chiang , Y. F. Chen, J. S, Lai and C. H. Tsai

Sun36: *Sex differences in antagonism of opioid antinociception,* A. Tseng, D. McNeil, M. S. Furness, K. Rice and R. Craft

Sun37: *Pain control for knee arthroscopy: peripheral effect of ketamine,* C. S. Wong and G. S. Huang

Sun38: *Delta-opioid receptor and (1DMe)NPYF-mediated effects in spinal antinociception,* M. Xu, K. Lemberg, P. Panula and E. Kalso

Signal Transduction Mechanisms

Sun39: *Desensitization of human delta opioid receptor increases phosphorylation of $G_{\alpha i}/G_{\alpha o}$ proteins,* S. Allouche, N. Marie and Ph. Jauzac

Sun40: *Impaired opioid receptor internalization following chronic morphine treatment,* H. Ammer, D. A. Eisinger and R. Schulz

Sun41: *Tyrosine phosphorylation of the kappa opioid receptor enhances agonist efficacy,* S. M. Appleyard, J. P. McLaughlin and C. Chavkin

Sun42: *On the mechanism of ERK regulation by mu opioid receptors in C6 glioma cells,* M. M. Belcheva, L. M. Bohn, P. Haas and C. J. Coscia

Sun43: *Mu opioid receptor spliced variant, MOR-1D, internalizes after morphine treatment in HEK-293 cells,* L. M. Bohn, L. S. Barak, C. Abbadie, Y.-X. Pan, G. W. Pasternak and M. G. Caron

Sun44: *Examination of several alternatively spliced mu opioid receptor (MOR-1) isoforms in functional assays,* E. A. Bolan, Y.-X. Pan and G. W. Pasternak

Sun45: *Involvement of MAPK pathways in the regulation of hippocampal opioid peptides expression induced by kainic acid,* S. S. Choi, J. K. Lee, M. R. Lee and H. W. Suh

Sun46: *Constitutive activity and adenylyl cyclase inhibition by mu and delta opioid receptors in HEK293T cells,* M. J. Clark and J. R. Traynor

Sun47: *Anatomical interaction between MOR and multiple G protein alpha subunits,* K. G. Commons, E. J. VanBockstaele, L. M. Kow, S. G. Beck and D. W. Pfaff

Sun48: *Ketamine potentiates morphine activation of the MAPK pathway,* I. Gomes, A. Gupta, M. Bansinath and L. A. Devi

Sun49: *μ-Opioid receptor activation of KIR3 is suppressed by the neurotrophin BDNF,* D. Ippolito, J. McLaughlin, S. Rogalski and C. Chavkin

Sun50: *The ERKSOME relationship between Src, MAPK and opioid receptors in signaling and regulation,* K. Kramer, M. L. Andria and E. J. Simon

Sun51: *A phosphatidyl-ethanolamine binding protein 23kDa-PEBP enhances $G_{i/o}$ protein mediated signalling of opioid receptors,* T. Kroslak, T. Koch, Z. Han, H. Rommelspacher and V. Höllt

Sun52: *Identification of GRK2 sites for agonist-stimulated δ opioid receptor phosphorylation and desensitization,* J. Guo, Y. Wu, W. Zhang, J. Zhao, L. A. Devi, G. Pei and L. Ma

Sun53: *Induction of multiple effects on adenylyl cyclase and CREB phosphorylation upon exposure to morphine of cells transformed with the μ-opioid receptor,* G. Mazarakou, M. Merkouris and Z. Georgoussi

Sun54: *Insulin potentiates opioid activity through tyrosine phosphorylation of the mu opioid receptor,* J. P. McLaughlin and C. Chavkin

Sun55: *Strategies for studying the roles of opioid receptor signaling,* E. Morou, A. Prombona and Z. Georgoussi

Sun56: *Opioid pretreatment increases cyclic GMP levels in SH-5YSY cells,* A. L. Parkhill and J. M. Bidlack

Sun57: *Differential interactions between opioid and α2 adrenergic agonists for enhancing [^{32}P]GTP azidoanilide (GTP-AA) incorporation into spinal $G_{o1\alpha}$,* S. C. Roerig and F. Karim

Sun58: *TrkB activation by BDNF inhibits the G protein gated inward rectifier KIR3 by tyrosine phosphorylation of the channel,* S. L. Rogalski, S. M. Appleyard, A. Patillo, G. W. Terman and C. Chavkin

Sun59: *The opioid-activated potassium channel (Kir 3) is inhibited by PLA2 generated eicosanoids,* S. L. Rogalski and C. Chavkin

Sun60: *Differential stimulation of [^{35}S]GTPγS binding by delta agonists at a TRP284 mutant of the human delta opioid receptor,* D. Stropova, Y. Hosohata, X. Li, R. Knapp, W. Roeske and H. I. Yamamura

Sun61: *Mu-, kappa$_3$- and ORL-1-receptor mediated activation of p42 and p44 mitogen-activated protein kinases in human neuroblastoma cells,* D. R. Thakker and K. M. Standifer

Sun62: *Unique interactions of SNC80 with the human delta opioid receptor (hDOR),* E. Varga, T. Okura, S. Cowell, Y. Hosohata, K. Hosohata, D. Stropova, W. R. Roeske and H. I. Yamamura

Sun63: *Characterizing the kinase PKU-α: a potential novel modulator of the mu opioid receptor,* J. Yates and L. Yu

Poster Session 2: (Monday, July 17, 13:30-15:30, Grand Ballroom)

Receptor Structure

Mon01: *Homodimerization of the μ-opioid receptor MOR1 in HEK 293 cells,* M. Händel, S. Schulz, T. Koch and V. Höllt

Mon02: *Identification of polymer, dimer and monomer forms of human kappa opioid receptor: involvement in receptor binding and activation,* A. Hasbi and J. M. Bidlack

Mon03: *Constitutively active mutants of the μ opioid receptor,* P. Huang, J. Li, C. Chen, W. Xu and L.-Y. Liu-Chen

Mon04: *Opioid receptors have complexes,* B. A. Jordan, I. Gomes, V. Nagy, N. Trapaidze and L. A. Devi

Mon05: *Identification of a membrane targeting domain in the C-terminus of the mu opioid receptor,* T. Koch, S. Schulz, M. Klutzny, E. Kahl and V. Höllt

Mon06: *Up-regulation of a constitutively active mutant of the rat μ opioid receptor by naloxone,* J. Li, P. Huang, C. Chen and L.-Y. Liu-Chen

Mon07: *Mutation of threonine 279 in the rat μ opioid receptor displays enhanced agonist affinity and substantial up-regulation after naloxone pretreatment,* T. G. Metzger, M. G. Paterlini, D. M. Ferguson and P. S. Portoghese

Mon08: *Differential expression of eight splicing variants are directed by a new promoter of the mouse mu opioid receptor gene (MOR-1),* Y.-X. Pan, J Xu, G. Rossi, M.-M. Xu, L. Mahurter, E. Bolan and G. W. Pasternak

Mon09: *Role of Ets-1 in the transcription regulation of mouse δ-opioid receptor,* P. Sun and H. H. Loh

Mon10: *Identification of residues in the putative seventh transmembrane domain of the human kappa opioid receptor exposed in the binding pocket,* W. Xu, J. M. Wang, J. K. de Riel and L.-Y. Liu-Chen

Mon11: *Study of S196A mutation of mu opioid receptor using knock-in strategy,* W. Yang, P.-Y. Law and H. H. Loh

Pharmacology of Opioids

Mon12: *Binding and internalization of fluorescent opioid peptide conjugates: visualization in living cells,* S. Arttamangkul, V. Alvarez Maubecin, G. Thomas, J. T. Williams and D. K. Grandy

Mon13: *Effects of opioids on cytokinetics in the germinal zone of the embryonic neocortex,* G. Bakalkin, K. F. Hauser, G. Nazarevskaja, Y. Trunova, T. Yakovleva and K. Reznikov

Mon14: *Comparisons of δ-mediated convulsions and antinociception in mice,* D. C. Broom, J. F. Nitsche, J. E. Pintar, J. H. Woods and J. R. Traynor

Mon15: *Intestinal opioid receptors (OR): coexpression of δ- and κ-OR in myenteric neurons,* D. R. Brown, S. Poonyachoti, A. Kulkarni-Narla and D. Townsend

Mon16 *Repeated kappa-opioid agonists alter uptake and synthesis of dopamine,* S. L. Collins and S. Izenwasser

Mon17: *Blockade of hyperalgesia to morphine by antisense oligodeoxynucleotides to $G_s\alpha$,* R. A. Cruciani and G. W. Pasternak

Mon18: *Acetylcholine / opioid interactions in acute pain,* M. I. Damaj and S. P. Welch

Mon19: *Receptor binding properties of ADL 8-2698, a potent opioid receptor antagonist,* R. N. DeHaven, J. A. Cassel, J. D. Daubert, D. Guo, E. K. Gauntner, V. Kumar and E. Mansson

Mon20: *Pharmacological profile of ADL 8-2698, a peripherally restricted opioid antagonist,* P. J. Little, S. L. Long, S. L. Gottshall, M. Koblish, D. Guo and D. L. DeHaven-Hudkins

Mon21: *Influence of aprotinin on plasma level of beta-endorphin and substance P during withdrawal in morphine-dependent rats,* I. Figurina, S. Sudakov, N. Terebilina, O. Medvedeva, I. Rusakova and M. Obrezchikova

Mon22: *Quantitative autoradiographic mapping of μ-, δ-, and κ-opioid receptors in the brain of δ-opioid receptor gene knockout mice,* R. J. Goody, D. Filliol, B. Kieffer and I. Kitchen

Mon23: *Anabolic-androgenic steroids – effects on opioid receptors and behavior,* M. Hallberg, P. Johansson, A. Kindlundh and F.Nyberg

Mon24: *Contribution of spinal novel μ-opioid receptor to endomorphin-2-induced antinociception,* T. Hayashi, S. Sakurada, J. E. Zadina, A. J. Kastin, A. Yonezawa, M. Takeshita, T. Fujimura, K. Murayama, C. Sakurada and T. Sakurada

Mon25: *Norbuprenorphine is a potent opioid agonist,* P. Huang, G. B. Kehner, L.-Y. Liu-Chen and A. Cowan

Mon26: *Possible involvement of morphine-insensitive mu_1-opioid receptors in the fentanyl-induced antinociception,* S. Imai, M. Narita, Y. Itou, Y. Yajima and T. Suzuki

Mon27: *The effect of P-glycoprotein on opiate transport and analgesia,* M. A. King, W. Su, A. H. Chang, A. Zuckerman, S. Bullock, S. P. Milo and G. W. Pasternak

Mon28: *Nonopioid motor effects of deltorphin analogs,* R. Lattanzi, E.Giannini and L.Negri

Mon29: *Characterization of [^{3}H]endomorphin 1 binding in rat brain membranes,* I. Lengyel, D. Biyashev, I. Szatmári, Zs. Canjavec, Cs. Tömböly, G. Tóth and A. Borsodi

Mon30: *Pharmacological characterization of SUPER DALDA, a potent μ-opioid analgesic,* C. L. Neilan, T. M.-D. Nguyen, P. W. Schiller and G. W. Pasternak

Mon31: *The action of delta analgesics in DOR-1 and enkephalin null mice*, J. F. Nitsche, K. C. Rice and J. E. Pintar

Mon32: *3-D isobolographic analysis of intrathecal mu opioid, alpha-2 adrenoceptor and 5-HT receptor mediated analgesia,* D. Paul

Mon33: *The antinociceptive effect of mirtazapine.* C. G. Pick, T. Rigai and S. Schreiber

Mon34: *(2S,3R)TMT-L-TIC-OH is a potent and selective antagonist at the δ opioid receptor in mice,* M. Rubenzik, K. Hosohata, J. Alfaro-Lopez, X. Tang, V. J. Hruby, W. Roeske and H. I. Yamamura

Mon35: *Differential involvement of spinal κ-opioid receptors in endomorphin-1-and-2-induced antinociception,* S. Sakurada, T. Hayashi, A. Yonezawa, L. Tseng, M. Narita, T. Suzuki, C. Sakurada and T. Sakurada

Mon36: *Down regulation of δ-opioid receptor in NG108-15 cells using NT-antibody*, S. K. Sharma, S. Imran and S. P. Singh

Mon37: *Presynaptic modulation by opioid receptors on excitatory and inhibitory transmission to rat subthalamic neurons,* K.-Z. Shen and S. W. Johnson

Mon38: *Low doses of morphine elicit acute thermal hyperalgesia in normal mice,* K. F. Shen and S. M. Crain

Mon39: *Mu, kappa, and delta opioid radioligand binding in amphibian brain,* C. W. Stevens, L. C. Newman and D. R. Wallace

Mon40: *Anti-pruritic effect of κ opioid receptor agonist TRK-820,* H. Umeuchi, T. Tanaka, K. Kawamura, K. Okano, T. Endo, J. Kamei and H. Nagase

Other Opioid Effects

Mon41: *Expression of novel guinea pig UGTs: effect of morphine on regulation in near-term placenta,* D. P. Andrews, S. R. Nagalla, G. D. Olsen and S. A. Smith

Mon42: *Increased weight and adiposity in mice lacking β-endorphin,* S. M. Appleyard, M. D. Hayward, J. I. Young, M. Rubinstein and M. J. Low

Mon43: *Propranolol decreases degradation of met-enkephalin-arg-phe across the heart,* B. A. Barron and E. B. Pearlman

Mon44: *Delta opioid receptor antagonist-mediated cell growth and apoptosis of human lung cancer cells,* Y. L. Chen, P.-Y. Law and H. H. Loh

Mon45: *Differential cardiorespiratory and analgesic effects of endomorphins 1 & 2,* M. A. Czapla and J. E. Zadina

Mon46: *SUPER DALDA enhances adrenergic responses in the dog,* M. Farias, K. Jackson, D. Yoshishige, H. H. Szeto and J. L. Caffrey

Mon47: *Verification of a μ opioid receptor model by Zn^{2+}-binding site engineering,* C. Fowler, I. Pogozheva, H. Akil, H. LeVine, III and H. Mosberg

Mon48: *Synergism of morphine and HIV-1 TAT protein promote toxicity in murine striatal neurons,* J. A. Gurwell, A. Nath, R. J. Goody, K. M. Martin, Y. Chen and K. F. Hauser

Mon49: *Opioids regulate oligodendrocyte survival through autocrine signaling,* K. F. Hauser, O. S. Itkis, B. A. Spruce and P. E. Knapp

Mon50: *Naloxone's effect on operant responding to food reward in the DBA/2 strain of mice,* M. D. Hayward and M. J. Low

Mon51: *Pharmacologic profiles indicate vagolytic opiate receptors in the S.A. node are delta specific,* K. Jackson, M. Farias, A. Goode and J. L. Caffrey

Mon52: *Role of opioids in hypothalamic control of GnRH release mechanism in ovariectomized rats,* G. Kaur

Mon53: *The anabolic-androgenic steroid nandrolone affects the dopamine receptors in the male rat brain,* A. Kindlundh, J. Lindblom, L. Bergström, J. Wikberg and F. Nyberg

Mon54: *Dynorphin $A_{1\text{-}13}$ and NMDA and thier effects on the HPA axis in fetal sheep,* L.Nardo, I. R.Young,, D.Walker and H. H. Szeto

Mon55: *Opioid agonist stimulated GTPγS binding in neonatal guinea pig brainstem,* A. Y. Matsuda and G. D. Olsen

Mon56: *Intracerebroventricular injection of anti-sense HuD oligonucleotides in mice results in severe motor dysfunction and seizures,* G. C. Rossi, D. T. Cannella, G. W. Pasternak and J. B. Posner

Mon57: *Naloxone fails to induce conditioned place aversion in mice lacking the mu-opioid receptor,* P.D. Skoubis, H. W. Matthes, B. L. Kieffer and N. T. Maidment

Mon58: *Motivational responses induced by morphine and cocaine in CB1 KO mice,* O. Valverde, M. Martin, C. Ledent, M. Parmentier and R. Maldonado

Mon59: *Effect of aluminium chloride on mice memory behavior and the long-term potentiation of rats hippocamal slices,* X.-R. Wu, X.-N. Zhu and R.-Z. Chen

Poster Session 3: (Wednesday, July 19, 13:30-15:30, Grand Ballroom)

Tolerance, Dependence and Addiction

Wed01: *Receptor desensitization as a possible mechanism of tolerance to mu opioids,* A.-A. Assi, W. Jin and C. Chavkin

Wed02: *The effect of opioids on Ca^{2+}/cAMP responsive element binding protein regulation,* W. Bilecki and R. Przewlocki

Wed03: *Resistance to morphine tolerance in mice lacking βarrestin-2,* L. M. Bohn, R. R. Gainetdinov, F. T. Lin, R. J. Lefkowitz and M. G. Caron

Wed04: *Preliminary studies of rebirthipe on naloxone-precipitated withdrawal symptoms in morphine-dependent mice,* Y. F. Chen, H. L. Fang, H. Y. Tsai, C. T. Hsu, Y. C. Lin and H. H. Loh

Wed05: *Potential role of RGS4 in desensitization of the mu opioid receptor on SH-SY5Y cells,* A. T. Crowder, H. B. Weems and T. E. Cote

Wed06: *Required treatment for substance abusers,* Ron Fagan

Wed07: *Serotonin transporter binding sites in fetal rhesus monkey brain: effect of gestational cocaine exposure,* Y. Fang and O. K. Rønnekleiv

Wed08: *Exploration of mechanism on low physical-dependence induced by dihydroetorphine,* Z.-H. Gong, D.-X.Wang and B.-Y. Qin

Wed09: *Naltrexone decreases gambling behavior,* J. E. Grisel and A. Shaw

Wed10: *Coupling of μ-opioid receptor to GIRK in EGFP-POMC neurons in the mouse,* N. Ibrahim, J. Smart, M. Rubinstein, M. J. Low and M. J. Kelly

Wed11: *Currents associated with the dopamine transporter in cultured dopamine neurons,* S. L. Ingram and S. G. Amara

Wed12: *Changes in AMPA receptors following morphine withdrawal in the rat brain,* C.-G. Jang, R. W. Rockhold and I.K. Ho

Wed13: *Mu opioid desensitization shares common mechanism with LTD in the hippocampal dentate gyrus,* W. Jin, A.-A. Assi, G. Terman and C. Chavkin

Wed14: *Effect of zinc on morphine analgesia, tolerance and dependence,* K. J. Kovács, A. K. Spartz and A. A. Larson

Wed15: *RB101(S)does not induce antinociceptive tolerance, or cross-tolerance with morphine,* S. Le Guen, F. Noble, M.-C. Fournié-Zaluski, B. Roques, J.–M. Besson and J. Buritova

Wed16: *GRK2 is the unique GRK involved in the desensitization of human delta opioid receptor,* N. Marie, S. Allouche, A. Hasbi and Ph. Jauzac

Wed17: *PKC-mediated inhibition of mu-opioid receptor internalization and morphine acute tolerance,* T. Matsumoto, M. Inoue and H.Ueda

Wed18: *Phosphorylation sites and regulation of the delta opioid receptor,* O. Maestri-El Kouhen, G. Wang, L. Erickson, P.-Y. Law and H. H. Loh

Wed19: *Imbalance in endogenous CCK/opioid systems and vulnerability to drug-taking,* F. Noble, J. L. Wilson, M. Mas Nieto, P. Coric and B. P. Roques

Wed20: *Dissociable effects of opiate withdrawal on drug-seeking (DS) and drug-taking (DT),* M. C. Olmstead and K. Hellemans

Wed21: *ERK inhibition reduces opioid tolerance in rats,* P. Pearson, G. Bishop, J. Trzaskos and H. Gutstein

Wed22: *Melanocortin receptor mediated effects in the alcohol preferring AA rats,* K. Ploj, E. Roman, A. Kask, P. Hyytiä, H. B. Schiöth, J. E. S. Wikberg and I. Nylander

Wed23: *Prior cocaine exposure in the fetal rhesus monkey increases striatal area cFOS expression,* O. K. Rønnekleiv, B. R. Naylor, M. A. Bosch and Y. Fang

Wed24: *Modulation of morphine-induced sensitization by δ-opioid receptor systems,* T. S. Shippenberg, W. Rea and A. C. Thompson

Wed25: *Changes in the intracellular distribution of brain mu opioid receptors and G-proteins associated with morphine tolerance,* M. Szûcs, G. Fábián, B. Bozó, M. Szikszay, G. Horváth and C. J. Coscia

Wed26: *Two mature forms of human nocistatin in brain and cerebrospinal fluid,* S. Tachibana, T.-L. Lee, F. M. Y. Fung, G. Zhang, F.-G. Chen, N. Chou, E. Okuda-Ashitaka, S. Ito, Y. Nishiuchi and T. Kimura

Wed27: *The role of glutamate release and nitric oxide on the mechanism of U-50,488 to prevent morphine tolerance,* P. L. Tao, H. C. Wu, C. C. Wu, C. H. Yang and C. C. Chou

Wed28: *NMDA receptor blockade mimics tolerance and sensitization to the locomotor effects of morphine,* K. A. Trujillo, K. P. Warmoth, D. J. Peterson, D. N. Albertson and R. M. Swadley-Lewellen

Wed29: *Maternal opiate exposure: long-term CNS consequences in the stress system of offspring,* I. Vathy

Wed30: *Adenoviral-mediated expression of the wild-type mu opioid receptor, the T394A mutant and the μ/δ receptor in neurons,* W. M. Walwyn, W. Z. Wei, C. W. Xie, A. M. Tan, H. W. Matthes, B. L. Kieffer and N. T.Maidment

Wed31: *Desensitization of mu/delta-tail chimeric receptors expressed in mouse DRG neurons,* W. Z. Wei, W. M. Walwyn, A. M. Tan, N. T. Maidment, H. W. Matthes, B. L. Kieffer and C. W. Xie

Wed32: *Operant discriminative stimuli, not classically conditioned stimuli, reinstates food-seeking,* I. A. Yun and H. L. Fields

Wed33: *Effect of SP_{1-7} on the dopamine D2 receptor mRNA in morphine dependent rat,* Q. Zhou, P. Le Grevès, A. Kindlundh and F. Nyberg

Nociceptin/Orphanin FQ and ORL1

Wed34: *Distribution of orphanin FQ/nocistatin transcripts in human immune cells,* J. Arjomand, S. W. Cole and C. J. Evans

Wed35: *Pain-inhibitory role of N/OFQ in the spinal cord,* M. Inoue and H. Ueda

Wed36: *Construction and characterization of constitutively active mutants of the human opioid receptor-like (ORL_1) receptor,* W. L. Kam and Y. H. Wong

Wed37: *Regulation of atrial natriuretic peptide by opioids in rats,* K. W. Kim, R. S. Woo, Y. P. Chung, Y. Son and K. P. Cho

Wed38: *Orphanin FQ/nociceptin attenuates morphine tolerance in rats using the hot plate test,* K. Lutfy, S. M. Hossain and N. T. Maidment

Wed39: *OFQ-induced modulation of LHPA axis activity: a neuroanatomical study,* M. A. Misilmeri, H. Akil and D. P. Devine

Wed40: *Direct identification of a peptide binding domain in the ORL1 receptor with [Bpa^{10}, ^{125}I-Tyr^{14}]-nociceptin,* L. Moulédous, C. M. Topham, H. Mazarguil and J.-C. Meunier

Wed41: *Cocaine sensitization increases the level of orphanin FQ-immunoreactivity in the rat hypothalamus,* S. Narayanan, H. Lam, N. T. Maidment and K. Lutfy

Wed42: *Orphanin FQ (OFQ)/nociceptin (N) and the C-terminal peptide of prepro OFQ/N are internalized by an amygdala-derived cell line (AR5) and SH5Y cells; OFQ/nociceptin (OFQ/N) internalization is modulated by μ and δ opioids,* M. J. Pellegrino, K. I. Fujimoto, J. W. Kasckow and R. G. Allen

Wed43: *The effect of maternal separation on tissue levels of nociceptin in rat brain,* E. Roman, K. Ploj and I. Nylander

Wed44: *Evidence for cross talk between ORL-1 and μ opioid receptors in human neuroblastoma cells,* K. M. Standifer, C. D. Mandyam and G. F. Altememi

Wed45: *Electroacupuncture analgesia in orphanin FQ (nociceptin) and opioid receptor-like receptor (ORL_1) knockout mice,* Y. Wan, B. Peng, J. Han and J. E. Pintar

Wed46: *Characterization of the human prepro-nociceptin gene and its regulation by cAMP*, N. T. Zaveri, C. J. Green and L. Toll

Medicinal Chemistry

Wed47: *An o-phthalaldehyde derivative of naltrindole as a fluorogenic affinity label,* B. Le Bourdonnec, R. El-Kouhen, P.-Y. Law, H. H. Loh and P. S. Portoghese

Wed48: *Endomorphin-substance P agonist chimeras,* S. F. Foran, I. Maszczynska, D. B. Carr, R. M. Kream, A. Misicka and A. W. Lipkowski

Wed49: *Biological properties of N-guanidyno opioid peptides,* A. W. Lipkowski, A. Misicka, I. Maszczynska, V. S. Hau, T. P. Davis and V. J. Hruby

Wed50: *Binding affinity at human cloned opioid receptors for a series of 3,4-dimethyl-4-(3-hydroxyphenyl)piperidines,* C. H. Mitch, D. O. Calligaro and J. S. Horng

Wed51: *R-ATC-ILE^{5,6} deltorphin II has partial agonist activity at the mouse delta opioid receptor,* S. M. Oakley, J. A. Gray, G. Toth, A. Bosodi and I. Kitchen

Wed52: *SUPER-DALDA: a polar, highly potent and selective mu opioid agonist,* P. W. Schiller, T. M.-D. Nguyen, I. Berezowska, S. Dupuis, G. Weltrowska, N. N. Chung and C. Lemieux

Wed53: *HS 378 – a new delta-selective and immunosupressive opioid antagonist,* H. Schmidhammer, R. Krassnig, H. Erlandsson Harris, T. Saxne, A. Kreicbergs and M. Spetea

Wed54: *In vivo role of prepro-OFQ/N160-187 in pain regulation,* H. Ueda, M. Inoue and R. G. Allen

Wed55: *An enzymatically stable kyotorphin analog induces pain in subattomol doses,* H. Ueda, M. Inoue, G. Weltrowska and P.W. Schiller

Late Addition

Wed56: *Modelling and sequence analysis of a novel opioid receptor from the zebrafish provides new insights into ligand-receptor interactions,* I. J. McFadyen, M. G. Paterlini, and D. M. Ferguson

Plenary Lecture I:

BETA ARRESTINS: TRAFFIC COPS OF CELL SIGNALLING

Robert J. Lefkowitz, Duke University, Durham, NC

ABSTRACT NOT PROVIDED

Plenary Lecture II:

MOLECULAR SUBSTRATES OF OPIATE ADDICTION

Eric J. Nestler. Laboratory of Molecular Psychiatry, Yale School of Medicine, New Haven, CT 06508.

Noradrenergic neurons of the locus coeruleus (LC), which mediate aspects of physical opiate dependence, have provided a useful model system in which to characterize molecular adaptations to chronic opiate administration. This dependence occurs at a cellular level and is mediated partly by upregulation of the cAMP pathway in LC neurons. Upregulation of this pathway, which occurs at the level of gene expression via regulation of the transcription factor CREB, is one mechanism driving activation of LC neurons during opiate withdrawal. Similar mechanisms contribute to opiate action in several other regions of the nervous system, including the nucleus accumbens (NAc) which is implicated in opiate reward. Upregulation of the cAMP pathway, including alterations in CREB, in the NAc has been related directly to changes in drug reward mechanisms. Opiates induce another transcription factor, ΔFosB, in the NAc, which also alters drug reward. This factor, due to its extraordinary stability, could mediate long-lasting changes in reward mechanisms related to addiction. Interestingly, several other drugs of abuse cause similar adaptations in the NAc, suggesting that these adaptations may be part of a common mechanism of addiction.

Plenary Lecture III:

SIGNALING PATHWAYS IN CELLS AND RETRO-VIRUSES INVESTIGATED BY STRUCTURAL BIOLOGY AND CHEMICAL APPROACHES

Bernard P. Roques, U266 INSERM, UMR 8600 CNSR, UFR des Sciences Pharmaceutiques et Biologiques, 4 avenue de l'Observatoire, 75270 Paris Cedex 06, France.

Determination by NMR, crystallography or computer modeling of the structure of protein targets is now currently used for studies about their mechanism of action at the molecular level and for de novo drug discovery. In some cases, domains of these proteins are accessible by solid phase synthesis. These strategies will be illustrated in the case of proteins involved in the signaling pathways as Grb2 and GAP and retroviral proteins, such as NCp7 and Vpr.

Goudrreau N. *et al.* **Nature Structure Biology,** 1994, 1(12), 898-907.
Cussac D. *et al.* **FASEB J.,** 1999, 13(1), 31-38.
Morellet N. *et al.* **EMBO J.,** 1992, 11, 3059-3065.
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Jacotot E. *et al.* **J. Exp. Med.** 2000, 191(1), 33-46.

Founders' Lecture:

1965 - 1975: A RENAISSANCE DECADE IN OPIATE RESEARCH

Brian M. Cox. Department of Pharmacology, Uniformed Services University of the Health Sciences, Bethesda, Maryland, USA.

The INRC was founded in 1969 in the midst of a decade of explosive growth in opiate drug research. Prior to the early 1960s, studies on opiate drug pharmacology were mainly of interest to neuropharmacologists devoted to finding new drugs for the treatment of pain. The majority of neuroscientists in this era (a small group by current standards) spent little time on opiates. During the ten years, 1965-1975, methadone maintenance therapy and other new approaches to the treatment of heroin addition were developed, and tentative steps towards our current understanding of the mechanisms of tolerance and dependence were made. Fentanyl, a novel potent opiate drug that has had a major impact on anesthetic practice was introduced, and naloxone, the first opiate antagonist without direct aversive activity, became available for the treatment of opiate drug overdose. During the same decade, it first became possible to measure binding of opiate drugs to their receptors and to picture the distribution of these receptors within the CNS. Receptors moved from being figments of the pharmacologist's imagination to quantifiable entities that could be related to pharmacologic actions, thus opening many avenues of research. The decade ended with the discovery of the endogenous opioid peptides and the sequencing of the enkephalins. These discoveries transformed our understanding of opiate drug actions and placed opioid researchers among those driving the expansion of neuroscience research over the following decade. INRC provided a unique forum for the presentation of many of these discoveries and a focus for the growth of research in this area.

S1-09:35

FACTORS CONTROLLING DRUG-SEEKING BEHAVIOUR FOLLOWING WITHDRAWAL FROM HEROIN AND COCAINE SELF-ADMINISTRATION.

T.J. De Vries, L.J.M.J. Vanderschuren, J.R. Homberg, E.H. Jacobs, A.B. Smit and A.N.M. Schoffelmeer. Research Institute Neurosciences Vrije Universiteit, Department of Pharmacology, Medical Faculty, Van der Boechorststraat 7, 1081 BT Amsterdam, The Netherlands.

High rates of relapse to drug use after prolonged drug-free periods characterize the behaviour of drug addicts. Besides drug-associated cues and stressful stimuli, an important event for provoking relapse to drug taking in humans is acute reexposure to the previously used drug. Since similar findings have been obtained in laboratory animals, stimulus-induced reinstatement of extinguished self-administration behaviour is generally accepted as an animal model representing some defining characteristics of relapse behaviour in humans. Our research program aims to elucidate the neurobiological mechanisms (at the molecular, cellular and system level) underlying such behaviour. Recently, we determined the effects of priming injections of direct and indirect dopamine (DA) agonists on heroin- and cocaine-seeking in order to study the proposed role of dopamine in ongoing addiction behaviour in more detail. Interestingly, all agonists that induced drug-seeking behaviour in our reinstatement paradigm also produced a sensitized response in a locomotor test and vice versa. These findings support the idea that the sensitizing properties of drugs of abuse may play an important role in the persistence of drug-seeking behaviour. Temporal changes and differences in the effects of direct and indirect DA agonists indicate a clear, but complex, involvement of DA in relapse behaviour. Temporal and persistent neuroadaptation of the mesolimbic dopamine system are thought to underlie these behavourial changes. Indeed, our *in vitro* neurochemical experiments show a gradual increase in depolarization-induced dopamine release from nucleus accumbens slices following repeated opiate exposure. Facilitated release of nucleus accumbens dopamine appeared a general neuroadaptation caused by all classical drugs of abuse, including morphine, alcohol, psychostimulants and nicotine, providing support for a common denominator view on drug addiction. Our studies on the molecular basis of these adaptations indicate that following withdrawal of heroin self-administration several gene-products involved in synapse formation and efficiency, growth and apoptosis are upregulated.

S1-10:00

CHRONIC EXPOSURE TO MORPHINE INITIATES NEW OPIOID RECEPTOR-COUPLED SIGNALING STRATEGIES.

A.R. Gintzler and S. Chakrabarti. SUNY Downstate Medical Center, Brooklyn, NY.

Multiple cellular adaptations are thought to underlie adaptation to chronic exposure to opioids. These include diminution of spare opioid receptors, decreased opioid receptor density and G protein content and coupling thereof. All of these putative mechanisms resonate with the view that opioid tolerance is a manifestation of a loss of opioid function, i.e., desensitization. Recent observations challenge the exclusiveness of this formulation and indicate that opioid tolerance also results from qualitative changes in opioid signaling as well. Adaptations to chronic morphine can also include altered consequences of opioid receptor coupling. These result from fundamental changes in the nature of effectors that are coupled to the opioid receptor-G protein-signaling pathway. Our studies indicate that chronic in vivo morphine results in the up-regulation of adenylyl cyclase isoforms of the type II family as well as a substantial increase in their phosphorylation state. Consequently, there is a shift in opioid receptor/G protein signaling from predominantly $G_{i\alpha}$ inhibitory to $G_{\beta\gamma}$ stimulatory. These adaptations to chronic morphine indicate the plasticity of opioid signal transduction mechanisms and the ability of chronic morphine to augment new signaling strategies, a predominant component of which is the G protein $G_{\beta\gamma}$ subunit. Thus, trafficking and availability of $G_{\beta\gamma}$ is a parameter that could be critical to some of the adaptations to chronic morphine.

S1-10:25

DIFFERNECES IN GENE EXPRESSION IN THE NUCLEUS ACCUMBENS FOLLOWING OPIATES.

J. Eberwine

ABSTRACT NOT PROVIDED

S1-11:20

FUNCTION OF THE MESOCORTICOLIMBIC CIRCUIT AT THE MILLENNIUM

S.J. Henriksen, The Scripps Research Institute, Depart. of Neuropharm. La Jolla, CA. www.scripps.edu/np/henriksen/

Multipolar cells of the ventral tegmental area of the brainstem contain GABA as their primary neurotransmitter (GA-VTA), and are intercalated between A10 dopamine-containing neurons. Evidence suggests that these cells influence dopamine neuron excitability and therefore dopamine release at more rostral sites e.g., in the ventral pallidum and pre-frontal cortex, regions critical for drug-related reinforcement. In addition, recent studies reveal that, in addition to local circuit regulation of dopamine processes, these long-projecting GABAergic neurons have discharge properties that correlate with reinforced behaviors. Interestingly, these projection GA-VTA neurons also exhibit discharge properties highly correlated to elevated arousal states. Both anatomical and physiological studies indicate these GA-VTA neurons have reciprocal connections with extensive regions of the brain including the prefrontal cortex, the basal forebrain, and the ventral pallidum, the caudal brainstem tegmentum and nuclei of the amygdala complex. These connections suggest an unappreciated integrative role for these GA-VTA neurons in the expression of motivated and rewarded behaviors, perhaps, in addition to their influence on dopamine mechanisms. We have investigated the role of these neurons both in drug self-administration, electrical brain stimulation, novelty, and in different arousal states. In combined single neuron in vivo recordings, combined with microinjection and encephalographic analysis, we have concluded that the fluctuation in spontaneous discharge of these high-gain GA-VTA neurons (20-200 Hz) is more closely associated with cortical arousal states than with specific behaviors. Our data suggests that these brainstem neurons and their proximal targets have an unrecognized role in facilitating sensory motor integration. My presentation will focus on the cellular correlates of motivated behaviors and will suggest that a re-evaluation of the relative roles of the reticular formation, thalamus, and basal forebrain in these behaviors, and arousal, is warranted.

S2-16:00

OPIATES AND THE GENOME

George Uhl, MD, PhD NIDA-IRP, Baltimore MD 21224

We can now place the six principal opioid genes into the context of a better-understood genome, and better understand the genes that are "upstream" and "downstream" to the functions of the neuropeptide and receptor opioid genes. "Downstream" genes are those whose expression patterns are regulated by activities at opioid gene products.

DNA microarray and other techniques suggest that a distinct fraction of the genes expressed in brain, less than 2%, change expression more than 2-fold following deletion of the mu opioid receptor, chronic morphine treatments, or chronic agonist treatments followed by withdrawal. These data generally confirm previous pictures that suggest alterations in a limited number of transcription factor, structural and regulatory genes' expression with limited adaptations, if any, in receptor and peptide gene expression. Advances in technologies suggest that nearly-complete lists of the genes regulated in these sorts of circumstances may well be available within the next 1-3 years.

"Upstream" genes are those whose activities alter expression of the three principal opioid peptide genes and the three principal opioid receptor genes. Many cis-acting elements capable of recognizing transcription factors are found in sequences tentatively identified as 5' flanking promoter/enhancer sequences for these six opioid family genes. Receptor genes generally contain housekeeping-like promoters, while the peptide genes generally contain TATA/CAAT boxes and the AP1-like features consistent with more active regulatory patterns. In vivo studies of the functions of several genes' promoters, especially that for proximal preproenkephalin 5' flanking elements, provides some assurance that several of the genes that are upstream to major in vivo regulatory processes have been identified. However, we understand little about the in vivo regulation of most of the opioid genes. Further elucidation of upstream and downstream genes can expand our understanding of opioid regulatory mechanisms, including those important for cell to cell differences in opioid function, human individual to individual differences in levels of gene expression, and gene-mediated contributions to day-to day-differences in the set points of opioid systems in the brain including the discoveries of the opioid genes themselves those responsible for tolerance, physical dependence and even addiction.

S2-16:10

GENES REGULATED BY μ AGONISTS AND μ DELETION: MICROARRAY STUDIES

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Studies of the patterns of altered gene expression induced by chronic morphine treatments and deletion of μ opiate receptor in knockout mice can elucidate regulatory mechanisms, including those possibly related to analgesia and opiate dependence. Analyses of hybridization of cDNA prepared from mouse brain mRNA to GeneChip Mu6500Affymetrix chips revealed positive hybridization signals for 30-40%. We compared data from control mouse brain cDNA to cDNA prepared from mRNA extracted from brains of μ opiate receptor knock-out mice and mice, wildtype mice chronically implanted with morphine pellets and those chronically implanted with placebo. 2% of the 6500 genes analyzed displayed gene expression changes, according to manufacturers' criteria, in both wildtype-knockout and morphine-placebo comparisons. Genes whose expression changed include transcriptional regulators, genes of neurotransmission, stress response genes, cytoplasmic regulators and second message pathway genes. These studies support the idea that gene expression changes could contribute to behavior changes and analgesic effects caused by morphine administration and by μ opiate receptor gene deletion. *Supported financially by the NIDA-IRP.*

S2-16:30

TRANSCRIPTIONAL REGULATION AND GENETIC VARIATIONS OF OPIOID RECEPTOR GENES

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Using electrophoretic mobility shift in combination with reporter gene assays data were obtained showing the involvement of a variety of transcription factors, such as AP-1, AP-2 and NFkappaB, in the transcriptional regulation of μ and δ opioid receptor genes. In addition, there is increasing evidence for allelic variations of opioid receptor and peptide genes. In the promoter region of the human prodynorphin gene, a polymorphic sequence which occurs as a single or tandemly repeated 68-bp element contains an AP1 factor binding site. Reporter gene assays showed an increase in the transcription efficacy with increasing number of the 68-bp elements. The μ opioid receptor gene contains a wide variety of allelic variations. In one polymorphism in the coding region serine 268 is exchanged to proline. This results in a retardation of receptor desensitization and a marked decrease in G protein coupling as measured by heterologous transfection of the receptor genes in frog oocytes and HEK293 cells. In the human δ receptor an allelic variation was found in codon 27 which results in an exchange of phenylalanine to cysteine. This mutation, however, had no substantial effect on binding and coupling of the receptor in oocytes or transfected HEK293 cells. Recently, also evidence for a post-transcriptional regulation of the human δ opioid receptor was found in tumor cell lines and melanoma tumors. Atypical mRNA processing resulted in a mRNA species in which 144 bp coding for the third cytoplasmic domain of the receptor protein were deleted. These data provide evidence for a high variability of opioid receptors and peptides occurring at the level of gene expression.

S2-17:20

TRANSCRIPTIONAL REGULATION OF MOUSE μ-OPIOID RECEPTOR (MOR) GENE

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MOR gene is over 53 kilobases long and its coding sequence is divided into 4 exons. Using deletional and transient transfection assays, a distal (D) and proximal (P) promoter of mouse MOR gene were identified in the cell lines endogenously expressing MOR. To determine the exact location of the promoter and to identify *cis*-DNA regulatory elements and *trans*-acting protein factors that are important for MOR gene promoter activity, we found that a MOR inverted (iGA) motif and a canonical Sp1 binding site are required for promoter activity. The transcription factors that bind iGA motif are SP1 and SP3. Recently, we found that a single-stranded (ss) *cis*-regulatory element and *trans*-acting protein factor are also important for proximal promoter activity. A 26 bp MOR polypyrimidine/polypurine region (PPy/u) interacts with a major nuclear protein, termed MOR polypyrimidine binding protein (mPy), that is not related to Sp factors. Southwestern blot analysis indicated that mPy protein is approximately 25 kDa in size. Functional analysis suggests that mPy protein can *trans*-activate MOR promoter as well as a heterologous promoter. Moreover, combinatorial activation of ss (mPy) and ds (Sps) DNA binding factors, interacting with an overlapping DNA (PPy/u) region, is necessary for proximal promoter activation. Thus, our results suggest that transcription of mouse MOR gene is regulated by an interplay of ss and ds DNA binding factors. (This work is supported by NIH-NIDA grants).

S2-17:45

A MOUSE MODEL OF SUBSTANCE ABUSE

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Inbred mouse strains differ markedly in their response to drugs of abuse and in their propensity to voluntarily consume drugs of abuse, including morphine, cocaine, and alcohol. A particularly extreme example, the C57BL/6J strain will voluntarily consume morphine to a level of ~200 mg/kg/day, leading to mortality in ~15% of the animals. In contrast, the DBA/2J strain will only consume morphine to a level of 10-20 mg/kg/day with no lethality. These strains also show differences in their responses to acute and chronic morphine exposure, and in their withdrawal symptoms. Quantitative trait locus (QTL) analysis has demonstrated that the majority of the variance in voluntary morphine consumption between these strains is due to a locus on chromosome 10, overlapping the μ-opiate receptor (MOR) gene. In order to confirm that sequence variants in the MOR gene are responsible for morphine drinking behavior we are crossing C57BL/6J, DBA/2J, and MOR knockout mice to produce the C57BL/6J MOR locus on a DBA/2J background, the reciprocal congenic, and a MOR knockout on C57BL/6J and DBA/2J backgrounds. These animals will be tested in the voluntary morphine consumption paradigm. Sequencing of the MOR gene in C57BL/6J and DBA/2J identified no differences in exonic sequences. We are now sequencing ~2 kb of genomic sequence 5' of the transcription start site, including core promoter and potential regulatory sequences. The binding sites for 5 transcription factors have been disrupted in C57BL/6J compared to DBA/2J. Recently, we have begun RNA profiling in these strains to identify pathways downstream of MOR. Successful elaboration of molecular pathways related to morphine response may reveal molecular targets for the development of therapeutics that will be useful for ameliorating drug addiction.

S2-18:10

USE OF CDNA MICROARRAYS TO ANALYZE GENE EXPRESSION RESPONSE TO OPIATES.

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cDNA microarrays allow the large scale measurement of gene expression levels of thousands of genes simultaneously. We have fabricated sets of human EST clones cDNA microarrays containing thousands of genes and are applying the arrays to address issues of cellular response to opiates. Human kidney fibroblast cell line 293 transfected with the murine mu opiate receptor is used as a model of cellular response to opiates. The culture cells are incubated with opiates with different signaling efficacies and abilities to regulate receptor trafficking then gene expression monitored by cDNA microarrays. The results and statistical analysis of opiate treatment will be presented. Generic application of cDNA microarray technology to tissue classification will also be discussed.

S3-09:30

THE CONTRIBUTION OF THE ANTI-OPIOID PEPTIDE CHOLECYSTOKININ (CCK) TO MORPHINE ANALGESIA AND TOLERANCE.

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The repeated administration of an opioid in the presence of specific environmental cues can induce tolerance specific to that setting (associative tolerance), while the repeated administration of an opioid in the absence of such environmental cues produces non-associative tolerance. Here we show that the anti-opioid peptide CCK acting at the CCK-B receptor is required for associative ($n=11$, $p<.01$) but not for non-associative ($n=8$) morphine tolerance in the rat. Additionally, we show that morphine administered in a morphine associated context increases Fos-like immunoreactivity in the basolateral ($n=4$, $p<.05$) and lateral amygdala ($n=4$, $p<.01$) and in hippocampal area CA1 ($n=4$, $p<.01$). Microinjection of a CCK-B receptor antagonist into the amygdala blocks the expression of associative morphine tolerance ($n=12$, $p<.01$). The CCK-B antagonist does not affect the expression of associative tolerance when microinjected into the VTA, nucleus accumbens, or area CA1. These results suggest that the development of associative tolerance to morphine involves a gradual compensatory increase in CCK or in the CCK-B receptor at the level of the lateral and basolateral amygdala.

S3-10:00

SYNAPTIC PLASTICITY IN THE PREFRONTAL CORTEX AND THE NUCLEUS ACCUMBENS: IMPLICATIONS FOR REWARD DIRECTED BEHAVIORS.

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The ability to extract motivationally relevant information from a complex environment, in order to form appropriate responses, is most essential to the survival of the organism. The mesocorticolimbic system comprised of prefrontal cortex (PFc), nucleus accumbens (Acb) and the ventral tegmental area has been implicated therein. The development of reward-predictive properties by initially neutral cues is one of the key factors in driving reward-seeking behavior. In order to understand what the contributions of the PFc neurons are in these associative learning processes, the firing of identified single units in the prelimbic area of the PFc (PL) of ten male rats was recorded with tetrodes over several sessions during various phases of a discrimination (Go\No-Go) task set in a Skinner box. In addition to neuronal correlates to reward delivery, CS-light and movement, 27% of all neurons recorded for multiple sessions developed sustained task related activity (STRA) during the time course of the learning phase. At first, these cells responded to reward delivery only. After acquisition of the task, modulation of responses had developed for the entire period of task execution, i.e., between CS-light presentation and reinforcer delivery. In the majority of these cells, the STRA disappeared following task reversal. This indicates that neurons in the PL are involved in associative learning as well as response flexibility and may form a neuronal basis of these functions. Synaptic plasticity within the afferent pathways to the PFC might serve as the mechanism by which this dynamic control can be exerted. Simultaneous recordings of PFc and its Acb projection area are presently being performed.

Support: HFSP, Van den Houten Foundation, NWO-MW grant 903-47-071.

S3-10:25

OPIOID-INDUCED SYNAPTIC PLASTICITY IN HIPPOCAMPUS. Charles Chavkin, Dept. of Pharmacology, Univ. of Washington, Seattle, WA 98195

The mechanisms underlying analgesic tolerance are multifaceted and likely to include receptor desensitization, compensatory adaptations within the nervous system, adaptive learning mechanisms, and changes in gene expression. At the cellular level, responses to opioids are readily observed, and prolonged activation of the opioid receptors leads to a gradual reduction in the amplitude of the evoked response. The mechanisms of this adaptive process were studied using the *in vitro* hippocampal slice preparation. As previously established, mu opioid receptor activation dramatically increases cellular excitability primarily by the reduction in the inhibitory GABAergic tone. Sustained exposure to either fentanyl or morphine resulted in the gradual reduction in the amplitude of the opioid response. Recent studies in this laboratory in collaboration with Drs. Lefkowitz and Caron, have shown that one component of the tolerance may be mediated by G protein receptor kinase phosphorylation of the mu opioid receptor. Hippocampal slices from GRK3 knockout mice show robust opioid responses, but the desensitization was significantly attenuated. The desensitization mechanisms are also likely to include NMDA-receptor dependent synaptic plasticity. NMDA receptor antagonists also attenuated the desensitization to sustained fentanyl. We found that fentanyl infusion produced long term depression (LTD). By reducing cellular excitability, LTD opposed the excitatory effects of opioid receptor activation. Since synaptic plasticity mediated by LTP and LTD may be cellular mechanisms for learning and memory, the effects of opiates on LTD suggest a mechanism for long lasting changes in opioid sensitive neuronal circuits that may contribute to drug dependence and craving.

S4-16:05

ENHANCED SPINAL NOCICEPTIN SYSTEM IS INVOLVED IN THE DEVELOPMENT OF MORPHINE TOLERANCE AND DEPENDENCE

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The effects of chronic treatments with morphine were examined using nociceptin/orphanin FQ (N/OFQ) receptor knock-out ($NOR^{-/-}$) mice and a novel nonpeptidic NOR antagonist (J-113397). This new compound revealed specific and potent antagonist activity for NOR in [^{35}S]GTPgammaS binding experiments using brain membranes, brain sections and insect cells expressing recombinant NOR and G protein subunits. J-113397 also found to be a pure antagonist in the peripheral nociception test in mice. The $NOR^{-/-}$ mice showed marked resistance to morphine analgesic tolerance without affecting morphine analgesic potency in tail-pinch and tail-flick tests. The $NOR^{-/-}$ mice also showed marked attenuation of morphine-induced physical dependence, manifested as naloxone-precipitated withdrawal symptoms following morphine treatments. Similar results were observed when J-113397 was administered to conventional ddY mice just before the assessment for tolerance and dependence. Morphine tolerance was significantly attenuated by intrathecal (1 nmol) or subcutaneous (10 and 30 mg/kg) injection of J-113397. This compound did not show any changes in the basal nociception threshold. Withdrawal symptoms were also inhibited by subcutaneous injection of J-113397 which had been given 60 min before naloxone-challenge, and there was no significant difference from the findings with $NOR^{-/-}$ mice. Furthermore, increased NOR gene expression in the spinal cord was observed during chronic morphine treatments for tolerance and physical dependence paradigms. Taken together, these findings suggest that NOR system induces important changes in plasticity observed upon morphine tolerance and dependence.

S3-11:20

MODULATION OF LTP AND LTD IN VTA AND N. ACCUMBENS IN VITRO BY AMPHETAMINE. J.A. Kauer, S. Jones, J.L. Kornblum, Y. Li. Dept. of Mol. Pharmacol., Brown University, Providence, RI.

Several lines of evidence suggest that glutamatergic synapses driving the dopamine reward system are modified during repeated exposure to addictive drugs. We examined rapid effects of amphetamine on synaptic plasticity at excitatory synapses onto dopaminergic neurons in the VTA onto n. accumbens neurons, using patch clamp recordings from brain slices. In the VTA, long-term depression (LTD) is induced by patterned activation of glutamatergic afferents. Amphetamine (1 μM) entirely blocked LTD, an effect prevented by 100 nM eticlopride and thus mediated through D2 receptors.

NMDA receptor-dependent LTP was described at excitatory synapses on n. accumbens neurons in response to high-frequency afferent stimulation. We found that 2.5 μM amphetamine blocks tetanus-induced LTP, though LTP can still be induced using low-frequency afferent stimulation paired with postsynaptic depolarization. After repeated exposure of rats to amphetamine [6 daily injections of 2.5 mg/kg, 7 days withdrawal], slices exhibited LTP. However, amphetamine exposure in vitro failed to block tetanus-induced LTP after amphetamine treatment in vivo. Our data demonstrate that synaptic plasticity at excitatory synapses in the reward pathway is profoundly altered by psychostimulant treatment. (supported by DA 11289)

S4-16:30

CHRONIC MORPHINE UP-REGULATES CELL SURFACE DELTA OPIOID RECEPTORS: IMPLICATIONS FOR PAIN CONTROL.

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Opioid receptors are known to undergo complex regulatory changes in response to ligand exposure. In the present study, we examined the effect of chronic morphine on the *in vitro* and *in vivo* expression and trafficking of δ opioid receptors (δOR). Prolonged (48h) exposure of primary neurons to morphine (10^{-6} M) resulted in a robust increase in the internalization of fluo-deltorphin, a highly selective fluorescent δ agonist. This effect was abolished when morphine was administered in the presence of a μ antagonist (CTOP), demonstrating that it was μ-specific. Western blotting and immunolabeling experiments revealed no significant change in overall δOR protein levels in neurons chronically treated with morphine as compared to untreated controls. However, subcellular localization of δOR using immunogold labeling revealed a significant increase (80-100%) in the proportion of receptors associated with the plasma membrane, indicating that prolonged μOR stimulation promoted membrane targeting of δOR from internal cellular pools. *In vivo* studies carried out in parallel demonstrated that chronic treatment of adult rats with morphine (3-10 mg/kg s.c. Q12h) similarly augmented targeting of δOR to neuronal plasma membranes in the dorsal horn of the spinal cord. Correspondingly, this treatment also markedly potentiated intrathecal D-[Ala^2]deltorphin II-induced antinociception. These findings suggest that prolonged treatment with μ-specific agonists such as morphine may lead to increased effectiveness of δ agonists, thereby opening new therapeutic strategies.

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S4-17:15

IDENTIFICATION OF A POPULATION OF PRIMARY AFFERENT NOCICEPTORS THAT DO NOT EXPRESS OPIOID RECEPTORS

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Primary afferent neurons, found in dorsal root and certain cranial nerve ganglia, are specialized to mediate various sensory modalities. Nociception is conferred to primary afferent neurons by the expression of members of 3 gene families: the ATP-gated ion channels (P2X receptors), the acid-sensing ion channels (ASICs) and the vanilloid receptors (VR1). Primary afferent nociceptors in rat exhibit one of two major phenotypes. Both phenotypes express VR1 and certain ASICS. Phenotype A neurons are likely to be vulnerable opioid-mediated presynaptic inhibition, since they express delta, mu and/or kappa receptors. In contrast, phenotype B neurons express an additional, pro-nociceptive gene product, the P2X3 receptor, and they do not express opioid receptors. Selective removal of the B phenotype neurons with a suicide toxin results in a complex pattern of transient and long lasting deficits in nociception. Supported by NIDA.

S4-18:05

DESCENDING FACILITATION IN OPIOID-INDUCED PAIN AND ANTINOCICEPTIVE TOLERANCE.

T.W. Vanderah, M.H. Ossipov, T.P. Malan, Jr., J. Lai and F. Porreca, Department of Pharmacology, University of Arizona HSC, Tucson, AZ 85724.

States of abnormal pain induced by injuries to peripheral nerves and opioid tolerance share common features including tactile allodynia and hyperalgesia of the hindpaws, decreased spinal opioid antinociception and loss of supraspinal/spinal opioid antinociceptive synergy. Modulation of pain by descending inhibitory and facilitatory systems from the rostral ventromedial medulla (RVM) are well known. This led us to study possible modulation of opioid-induced pain and tolerance by RVM pathways. In s.c. morphine (MS), -but not placebo-, implanted rats, tactile allodynia and hyperalgesia were observed; this abnormal pain was blocked by RVM injection of lidocaine suggesting tonically active descending facilitation. The i.th. MS antinociceptive dose-response curve (DRC, tail-flick) was displaced to the right in MS-pelleted rats, indicative of tolerance; this right shift in the DRC was blocked by RVM lidocaine in a time-related manner. Similarly, the s.c. MS antinociceptive DRC was displaced to the right in MS pelleted rats and this right shift was also blocked by RVM lidocaine, suggesting restoration of supraspinal/spinal antinociceptive synergy. As animals implanted with MS pellets displayed hyperalgesia, the experiments were repeated by normalizing the foot-flick threshold to control levels by reducing the stimulus intensity. At predurg baseline responses elicited by lower stimulus intensity, the i.th. morphine DRC (foot-flick) in MS pelleted rats was not different from the placebo group. The data suggest that chronic opioids increase pain which requires a higher opioid dose at the spinal level accompanied by a loss of supraspinal/spinal opioid synergy. Increased pain and opioid tolerance appear to result from tonic activity of descending facilitation from the RVM offering novel approaches to limit opioid "tolerance".

S4-17:40

MODULATION OF REWARDING EFFECTS OF OPIATES BY NOCICEPTIVE STIMULI

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Clinical studies have demonstrated that when opiates are used to control cancer pain, psychological dependence and analgesic tolerance are not a major concern. The present study was, therefore, designed to investigate the modulation of rewarding effects of opiates under inflammatory chronic pain in SD rats. Formalin (2.5%, 50μl) or carrageenan (1%, 100μl) was injected into the plantar surface of the rat paw. Formalin and carrageenan reduced the paw pressure threshold. The hyperalgesia lasted for 9 to 13 days. Morphine produced a significant place preference. This effect was significantly attenuated in inflamed groups as compared with the respective non-inflamed groups. Furthermore, the morphine-induced place preference in the inflamed group gradually recovered to the respective control level as the inflammation healed. To elucidate the mechanism of this attenuation, the effects of pretreatment with κ-opioid receptor antagonist nor-binaltorphimine (nor-BNI) on the development of the morphine-induced place preference under inflammation were examined. Nor-BNI eliminated the suppression of the morphine-induced place preference in inflamed groups. The morphine-induced increase in dopamine turnover in the limbic forebrain was suppressed under inflammation, and the suppression was abolished by the pretreatment with nor-BNI. These results suggest that endogenous κ-opioid systems may be activated by chronic inflammatory nociception, resulting in the suppression of the development of rewarding effects produced by morphine.

S5-09:35

CONSEQUENCES OF OPIOID RECEPTOR PHOSPHORYLATION.

Odile Maestri-El-Kouhen, Horace H. Loh and P.Y. Law. Department of Pharmacology, University of Minnesota, Minneapolis, MN, 55455 U.S.A.

Agonist-induced phosphorylation of the opiod receptors have been well established. Several studies have suggested that the rapid phosphorylation of the opioid receptor is the mechanism for the desensitization of the receptor. However, using the inhibition of intracellular cAMP production as the readout, we were unable to correlate the receptor phosphorylation and desensitization induced by agonist. Over-expression of the cellular components that are involved in receptor desensitization, GRK and β−arrestin2, resulted in an increase in the magnitude of receptor phosphorylation but not the rate of desensitization. Since the opioid receptor is coupled to the adenylyl cyclase efficiently, the failure to observe rapid desensitization could be due to the high level of receptor being expressed. Using the ecdysone-inducible expression system, we were able to control the δ-opioid receptor's level in HEK293 cells with ponasterone A. It could be demonstrated that the rate of agonist-induced receptor desensitization was dependent on the receptor's level. Further, the rapid desensitization of the δ-opioid receptor involves both the phosphorylation and the internalization of the receptor. By generating various receptor mutants, we have determined that agonist-induced receptor phosphorylation occurs only at the carboxyl tail domain of the δ-opioid receptor, and two of the Ser/Thr residues within this domain are phosphorylated. The sequential phosphorylation of these two sites induced by agonist, and their roles in the uncoupling and internalization of the receptor will be discussed in details during the presentation.

S5-10:00

MECHANISMS OF AGONIST-INDUCED DOWN-REGULATION OF THE HUMAN κ OPIOID RECEPTOR

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Previously we showed that the human κ opioid receptor (hkor) stably expressed in CHO cells underwent down-regulation following U50,488H treatment. In the present study, we determined the mechanisms underlying this process. U50,488H caused a significant down-regulation of the hkor, while etorphine did not. Neither U50,488H nor etorphine caused down-regulation of the rat κ opioid receptor. Expression of the dominant negative mutants arrestin-2(319-418) or dynamin I-K44A significantly reduced U50,488H-induced down-regulation of the hkor. Co-expression of GRK2 or GRK2 and arrestin-2 permitted etorphine to induce down-regulation of the hkor, while expression of arrestin-2 or dynamin I alone did not. Expression of the dominant negative mutants rab5A-N133I or rab7-N125I blunted U50,488H-induced down-regulation. Pretreatment with lysosomal enzyme inhibitors (EST or chloroquine) or proteasome inhibitors (Proteasome Inhibitor I, MG-132 or lactacystin) decreased the extent of U50,488H-induced down-regulation. A combination of chloroquine and Proteasome Inhibitor I abolished U50,488H-induced down-regulation. These results indicate that U50,488H-induced down-regulation of the hkor involves GRK-, arrestin-2-, dynamin-, rab5- and rab7-dependent mechanisms and receptors appear to be trafficked to lysosomes and proteasomes for degradation. To the best of our knowledge, these results represent the first report on the involvement of rab5 and rab7 in agonist-induced down-regulation of a G protein-coupled receptor.

S5-10:25

RELATIONSHIP OF LIGAND-INDUCED ERK ACTIVATION AND MU OPIOID RECEPTOR PHOSPHORYLATION

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Activation of extracellular signal regulated kinases (ERK1/2) by DAMGO indicated that ERK pathway could be important for opioid receptor signaling. The involvement of the ERK pathway in mu opioid receptor phosphorylation and desensitization has recently been reported. We hypothesized that receptor phosphorylation through the ERK pathway may influence the receptor function. In CHO cells stably expressing the mu opioid receptor and transfected with a constitutively active MKK1 mutant to specifically activate ERK, we demonstrated that the mu opioid receptor is not a substrate for ERK. Inhibitors of MKK and phosphatidyl inositol-3 kinase (PI3K) blocked DAMGO-induced ERK activation, but showed no significant effect on mu receptor phosphorylation. Furthermore, transfection with dominant negative Ras cDNA was not able to prevent DAMGO-induced ERK activation or mu receptor phosphorylation. These results suggest that the ERK pathway is involved in mu opioid receptor signaling through a Ras-independent mechanism involving PI3K but has no direct regulation of mu receptor phosphorylation.

S5-11:20

SIGNIFICANCE OF EXCITATORY Gs-COUPLED OPIOID RECEPTOR FUNCTIONS IN VIVO

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Electrophysiologic studies of opioid effects on nociceptive types of sensory neurons in culture indicated that excitatory Gs-coupled, GM1 ganglioside-regulated opioid receptor functions may play significant roles in attenuating analgesia and exacerbating tolerance/ dependence by masking inhibitory Gi/Go-coupled opioid receptor-mediated effects (Crain & Shen, TiPS '90, '98). These in vitro studies guided formulation of novel behavioral tests in mice demonstrating that selective antagonism of excitatory opioid receptors by ultra-low doses of naltrexone: 1) blocks low-dose-morphine-induced hyperalgesia in normal mice, unmasking potent opioid analgesia (S & C, INRC '00); 2) markedly increases the analgesic potency of higher doses of morphine; 3) attenuates opioid tolerance and withdrawal-jumping effects after chronic morphine treatment (C & S, PNAS '95, TiPS '98). Furthermore, recent clinical studies of pain patients cotreated with ultra-low doses of naloxone of nalmefene show significant enhancement of morphine's analgesic potency, as predicted by our preclinical studies in vitro and in vivo (C & S, PAIN '00; see also INRC '00 Abstr. by Sherman et al).

S7-09:35

NIDA MEDICATIONS EFFORTS IN DEVELOPING TREATMENTS FOR OPIATE & STIMULANT DEPENDENCE

F. Vocci, National Institute on Drug Abuse, Bethesda, MD

NIDA medications efforts aimed at developing medications for opiate dependence have concentrated on the development of levomethadyl acetate (LAAM; approved in 1993) and buprenorphine and buprenorphine/naloxone (BUP products). The BUP products have progressed to "approvable" status and FDA approval is anticipated by the end of this calendar year. These additional medications will give the clinician a spectrum of agents from full agonist to partial agonist to competitive antagonist (naltrexone). Farther back in the opiate dependence treatment pipeline, depot naltrexone, dextromethorphan, and lofexidine are in clinical development, while kappa opioid antagonists and CRF antagonists are under preclinical evaluation. Two approaches have been used to seek and test medications for the treatment of cocaine dependence. The first approach, called "top-down", tests currently available medications as putative treatment medications in clinical trials. Disulfiram, desipramine, and selegiline are medications that have some evidence of efficacy; confirmatory clinical studies are underway or being planned. The second approach, "bottoms up", seeks to identify and develop novel medications based on ability to modulate effects of cocaine. Using this approach, biogenic amine transport inhibitors have been advanced to clinical testing and selective agonists for specific dopamine receptor subtypes are being advanced to preclinical and clinical development. More recently, a conceptual shift has occurred and the program is refocusing on discovery and testing of medications that modulate processes involved in the propensity to relapse: e.g., cueing, priming and stress-induced increases in cocaine intake. Inasmuch as these processes may be involved in all drug addicted states, the program is evolving towards medications as relapse prevention agents for all drug dependencies.

S7-10:00

EVALUATION OF OPIOIDS FOR THE TREATMENT OF COCAINE ABUSE

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Opioids acting at mu, kappa and delta opioid receptors may modulate the activity of neural substrates that mediate the abuse-related effects of cocaine. Accordingly, we studied the ability of mu, kappa and delta opioid agonists to modify the reinforcing effects of cocaine in a drug self-administration model in rhesus monkeys. Monkeys were trained to self-administer cocaine (0.032 mg/kg/inj) and 1 gram food pellets under a second-order schedule of reinforcement that permitted up to 80 drug injections per day and 100 food pellets per day. Tests were conducted for a period of 7 consecutive days. During each test, either saline or one of several unit doses of cocaine (0.0032-0.1 mg/kg/inj) was made available for self-administration, and either saline or a dose of a mu, kappa or delta agonist was administered by continuous infusion. Drugs acting at all 3 opioid receptors dose-dependently decreased cocaine self-administration. However, opioids differed in the degree to which they also produced undesirable side effects such as decreases in food-maintained responding. The most selective compounds were low efficacy mu agonists, such as nalbuphine, and benzomorphan kappa agonists with mixed activity at both kappa and mu receptors, such as ethylketocyclazocine. (Supported by Grants DA KO5-DA00101, P50-DA04059, RO1-DA02519 and RO1-DA11460 from NIDA)

S7-10:25

DISCONNECTING PAIN FROM THE BRAIN WITH LIGAND TARGETED CYTOTOXINS.

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The ability to make targeted cell deletions provides a powerful tool to study nervous system function and dissect specific neural circuits in vivo. The techniques range from photodeletion of dye labeled cells, to toxic catecholamine derivatives, to expression of suicide genes in transgenic animals. The ligand-targeted cytotoxin approach provides a versatile, modular method for cell deletion because a variety of ligands can be conjugated to a cytotoxin. Toxins come from plants (saporin) or bacteria (diphtheria, pseudomonas, anthrax), usually the native binding domains are replaced with the targeting ligand. To target and kill cells expressing substance P/NK1 receptors, novel conjugates were generated between derivatized substance P (SP) and a truncated Pseudomonas exotoxin (PE35). This toxin stops protein synthesis when it escapes from the endosome and kills the cell over the course of 24–48 hrs. Intrathecal infusion of as little as 15 pmol of SP-PE deleted NK1 receptor-expressing cells from the dorsal spinal cord and caused regionally selective loss of thermal *and* mechanical pain yet other somatosensory and locomotor abilities were retained. Intrastriatal injections caused a zone of NK1 positive neuronal loss without affecting other cells in the vicinity. Opioid peptides have also been attached to cytotoxins. These data demonstrate the remarkable selectivity and utility of the peptide ligand targeted cytotoxins.

S7-11:20

HOW DOES NOCICEPTIN BIND AND ACTIVATE THE ORL1 RECEPTOR?

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Although nociceptin (noc) and the ORL1 receptor share high sequence similarity with dynorphin A (dyn) and the κ-opioid receptor (KOR1), respectively, the two peptides use distinct molecular pathways to bind and activate their cognate receptors. Based upon the different functional architectures of noc and dyn, the *in vitro* pharmacological properties of dyn/noc hybrid peptides, such as in particular dyn[1-5]-noc[6-17], and of ORL1/KOR1 chimeric receptors, it appears that activation of KOR1 by dyn requires interactions of its N-terminal tetrapeptide (Y^1GGF) with the receptor opioid binding pocket, located within the transmembrane helix bundle, whilst noc may activate the ORL1 receptor through interactions of its positively charged core (R^8KSARK) with the negatively charged second extracellular receptor loop.

These mechanistic aspects will be illustrated with reference to a 3-D molecular model of the ORL1 receptor and its complex with nociceptin (1), and its further experimental validation through the use of photoreactive (Bpa) and environment-sensitive fluorescent (NBD) noc derivatives.

(1) C.M Topham, L. Moulédous, G. Poda, B. Maigret and J .-Cl. Meunier (1998) Protein Eng. **11**, 1163-1179.

S8-09:05

OLIGOMERIZATION OF DOPAMINE AND SEROTONIN RECEPTORS: INSIGHTS INTO RECEPTOR STRUCTURE AND TRAFFICKING

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G protein-coupled receptor signal transduction mechanisms have been modeled with the assumption that only monomeric receptors participate in these processes. However, since we and others have documented that GPCRs form dimers and oligomers, a re-evaluation of the mechanisms thought to be involved in GPCR function is required. Using mutant receptors, we present evidence that GPCRs function only as oligomers in the cell. Thus far, little is known about the structural basis of the receptor-receptor interactions. We show dimers form the building blocks of the oligomers. Using dopamine and serotonin receptors, we demonstrated that agonist treatment appears to stabilise the receptor oligomers. An investigation of the structural assembly involved in oligomerization showed that there are several types of interactions including hydrophobic transmembrane domain interactions and intermolecular disulphide bonds that appear to be of varying predominance in different receptors, even those of closely related subtypes. We have also shown that closely related receptors hetero-oligomerize and these novel interactions set the stage for novel functions as a result of these associations.

S8-09:30

A ROLE FOR DIMERIZATION IN OPIOID RECEPTOR CROSS-TALK.

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Protein-protein interactions are involved in the regulation of a number of biological processes. It is well established that a variety of cell surface receptors interact with each other to form dimers and that this is essential for their activation. A variety of G-protein coupled receptors including members of the rhodopsin, secretin and metabotropic glutamate receptor family have been recently shown to exist as dimers. In a number of cases dimerization alters ligand binding, signaling and trafficking properties. We have examined the dimerization of opioid receptors. Delta and kappa receptors exist as homodimers with distinct physical and functional properties. These receptors are able to heterodimerize with each other and with other members of the G-protein coupled receptor super family. Heterodimerization between opioid receptor types (kappa/delta and mu/delta) leads to changes in agonist affinity, efficacy and/or potency. Recently we have examined dimerization between opioid receptors and other members of the rhodopsin family. When co-expressed in heterologous cells, these receptors interact with each other to form SDS-stable dimers/oligomers; dimerization leads to modulation in their signaling as well as trafficking properties. Thus, dimerization appears to be an universal phenomenon that provides a mechanism for cross-talk between opioid and other G-protein coupled receptors.

This work is supported by grants DA08863 and DA00458 (to L.A.D).

S8-09:55

BIVALENT LIGANDS AS PROBES TO EVALUATE OPIOID RERCEPTOR DIMER MODELS

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Ligands having two pharmacophores connected by a spacer (bivalent ligands) have the potential for bridging vicinal recognition sites on neighboring receptors. For entropic reasons, such bridging may be manifested by a substantial increase in potency and affinity when the spacer is of optimal length. Early studies (1982) with bivalent ligands containing opioid agonist and antagonist pharmacophores have suggested the presence of opioid receptor dimers. In view of recent reports on dimers among G protein-coupled receptors in general, and opioid receptors in particular, we have modeled dimeric opioid receptors based on evidence for swapping of TM5,6 and TM1-5 domains of monomers in the dimeric state. Based upon our model and protection studies with β-FNA, the two recognition sites on the dimeric μ opioid receptors are proposed to be negatively coupled. The possible implications of this model with respect to the recognition sites for agonists and antagonists will be discussed.

O1-11:45

HIPPOCAMPAL LTP IS SUPPRESSED AND BECOMES DRUG-DEPENDENT AFTER CHRONIC EXPOSURE TO OPIATES

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Hippocampal long-term potentiation (LTP) is considered as a leading experimental model for the synaptic changes underlying learning and memory. Recent studies from many laboratories including ours have demonstrated that inhibition of NMDA receptors or Ca^{2+}/Calmodulin dependent kinase, two important molecules in the formation of LTP, would lead to significantly attenuation of the opiate dependence. Measurement of LTP in the CA1 synapse after chronic administration of opiates such as morphine and heroin has been further carried out to test the potential opiate effect. The results showed that hippocampal LTP was significantly suppressed during the withdrawal period after chronic opiate treatment in both freely moving and urethane-anaesthesia rats while it was not affected by acute exposure to opiates. The suppression of LTP was already significant in rats receiving opiates for 5 days and reached to the maximal after 10-day opiate treatment. The capacity for LTP was slowly recovered to the apparent normal level about 72 hours after discontinuation of 10-day opiate treatment. In addition, the capacity for LTP could be restored to the normal during the withdrawal period by re-exposure of opiates, or by application of inhibitors of cAMP-dependent protein kinase A but not by that of protein kinase C. The suppression of hippocampal LTP by chronic opiate treatment was also functionally confirmed by water maze task. Thus, our results demonstrated that chronic opiate abuse could significantly modify the synaptic plasticity in hippocampus that may play an important role in the development of drug dependence.

O1-12:00

MULTIPLE CELLULAR MECHANISMS OF OPIOID WITHDRAWAL

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The midbrain periaqueductal gray (PAG) is involved in the expression of many signs of opioid withdrawal. We examined the effect of chronic morphine treatment on PAG neurons in brain slices from C57B16/J and Balb/C mice, using patch clamp techniques. When PAG slices were maintained in morphine (5μM), addition of naloxone (1μM) increased the rate of miniature GABAergic synaptic currents in PAG slices from morphine dependent, but not vehicle animals. In slices from vehicle animals, met-enkephalin (ME, 30μM) induced an outward current (K_{ir}) which reversed polarity near E_K. In spontaneously withdrawn slices from dependent animals, ME also inhibited a non-selective cation current. The ME induced K_{ir} current in brain slices was reduced in morphine dependent animals, compared to vehicle animals. Similarly, mu-opioid inhibition of calcium channel currents (I_{Ca}) was reduced in acutely isolated PAG neurons from morphine dependent animals. Chronic morphine treatment appears to alter mu-opioid receptor coupling in mouse PAG by (1) increasing coupling to presynaptic inhibition in GABAergic terminals, (2) inducing coupling to a postsynaptic cation current, and (3) reducing coupling to postsynaptic K_{ir} and I_{Ca} currents.

O1-12:15

CHRONIC MORPHINE TREATMENT ALTERS NUCLEUS ACCUMBENS NMDA RECEPTOR PROPERTIES

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Previously, we reported that chronic morphine altered NMDA receptor–mediated synaptic transmission in the nucleus accumbens (NAcc) that may involve a change in NMDA subunit composition. To test the hypothesis, we studied the effects of chronic morphine treatment on the electrophysiological properties of NMDA receptors in freshly-isolated NAcc neurons. We assessed the affinity of various NMDA agonists and antagonists known to discriminate between different NR1/NR2 heteromers. Chronic morphine had little effect on currents induced by glutamate and homoquinolinic acid, compounds able to discriminate betweeen NR2A/B and NR2C. However, the effect of drugs (glycine, 7-Cl-kynurenic acid and Ifenprodil) that discrimate between NR2A and NR2B were markedly altered. Chronic morphine also reduced the Mg^{2+}-mediated block of NMDA receptors, an effect usually associated with NR2C expression. We also studied NMDA receptor mRNA subunit expression with single-cell RT-PCR and found no qualitive difference of NR2A/B and C subunit expression between placebo and morphine-treated rats. These results suggest that chronic morphine probably increases NR2A/NR2B ratio in NAcc neurons. Functionally, such NR2A overexpression might decrease NAcc neuronal excitability by inducing a faster deactivation rate for NMDA receptors as observed in a NAcc slice preparation (Martin et al., J.Neurosci. 19:908,1999).

O2-18:30

SINGLE-NUCLEOTIDE POLYMORPHISMS IN THE HUMAN MU OPIOID RECEPTOR GENE ALTER RECEPTOR-CAM BINDING AND G PROTEIN COUPLING

D. Wang, J.M. Quillan & W. Sadée. Department of Biopharmaceutical Sciences, University of California San Francisco, CA 94143-0446.

Calmodulin (CaM) has been proposed to bind to the third intracellular loop of the mu opioid receptor (MOR). CaM appears to compete with G protein for overcapping binding domains in the i3 loop of MOR. Thus binding of CaM to MOR suppress basal G protein coupling of MOR. Upon receptor activation, CaM appears to dissociate, thereby permitting G protein coupling to proceed. Moreover, CaM may serve as a second MOR messenger *per se*. Mutant MOR lacking CaM binding display distinct signaling pathway as judged by cDNA expression array analysis. Single-nucleotide polymorphisms (SNP) have been found previously in *hMOR* gene that lead to single amino acid mutation in the third intracellular loop (namely R260H, R265H and S268P). In this study, we tested whether these SNP variants affect CaM binding and G protein coupling. The results showed that R265H and S268P mutant hMOR have decreased CaM binding affinity compared to wild-type hMOR, while all three mutants have varying defect in G protein coupling. These results demonstrate substantial changes in the signal transduction of MOR and variant MOR receptor carrying human SNP's. Supported by NIDA grant DA 04166

O2-18:45

GENE EXPRESSION PROFILES IN ACUTE MORPHINE DEPENDENCE AND WITHDRAWAL.

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To understand the molecular basis of withdrawal from self and passive administration of opiates, we evaluated gene expression profiles in male Swiss Webster mice in which we induced acute morphine dependence with a sustained release preparation and precipitated withdrawal with Naloxone. Animals treated with vehicle, morphine, naloxone or morphine and naloxone were sacrificed at 6, 13 and 24 hours after treatment, and total RNA (10ug) isolated from whole brain and specific brain regions was used to screen custom mouse microarrays (5,200 cDNA's and opioid controls) printed on glass. Comparison of global gene expression patterns to various specific brain regions showed more unique clusters in hypothalamic–striatal regions. MAP kinases and δ receptors that were up-regulated in the acute phase of morphine effect (6 hours) were down-regulated after naloxone challenge. Time-course analyses indicate global up-regulation of gene expression one hour following naloxone challenge and at 13 hours following morphine treatment. We will present models of regulatory pathways involved in acute morphine dependence and withdrawal.

Supported by NIH grant to SRN (DA11318).

O3-11:45

CHRONIC MORPHINE INDUCES SYNAPSE-SPECIFIC CHANGES IN THE NUCLEUS ACCUMBENS

J.M. Brundege, J.T. Williams. Vollum Institute, Oregon Health Sci Univ, Portland OR.

The nucleus accumbens is a brain region implicated in the motivational aspects of drug abuse. However, our understanding of how chronic drug use alters synaptic transmission in this area is limited. The present study examined the effects of chronic morphine treatment on excitatory postsynaptic currents (EPSCs) and inhibitory postsynaptic currents (IPSCs) in accumbens shell and core medium spiny neurons. The mixed μ and δ opioid receptor agonist [Met]^{5}enkephalin inhibited, and forskolin, an activator of adenylyl cyclase, potentiated synaptic currents at all four synapses tested. Chronic morphine treatment enhanced the effects of [Met]^{5}enkephalin on EPSCs in the shell, while IPSCs in the shell and both EPSCs and IPSCs in the core were unchanged. Likewise, chronic morphine treatment selectively enhanced the effects of forskolin on EPSCs in the shell, without altering the effects of forskolin at the other three synapses tested. Chronic morphine treatment thus selectively altered the regulation of synaptic activity by opioids and cAMP in excitatory synapses of the shell. These data demonstrate that a) the shell of the nucleus accumbens is subject to specific synaptic alterations after chronic morphine treatment that do not occur in the core, and b) that there is further synapse-specificity of these alterations within the shell. These changes in synaptic transmission may play an important role in mediating the motivational aspects of drug addiction and withdrawal

O3-12:00

TARGETING OF MU-OPIOID RECEPTORS IN VENTRAL TEGMENTAL AREA NEURONS THAT PROJECT TO MEDIAL PREFRONTAL CORTEX

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Activation of mu-opioid receptors (MOR) in the ventral tegmental area (VTA) inhibits GABA neurotransmission, yet is not known if this inhibition directly modulates activity in the medial prefrontal cortex (mPFC). We used electron microscopic immunocytochemistry and retrograde tract-tracing to determine if neurons in the VTA that project to the mPFC contain MOR. Rats received injections of the tracer fluoro-gold (FG) into the mPFC, and tissue sections throughout the VTA were processed for ultrastructural examination of FG and MOR. Peroxidase labeling for FG was present in VTA cell bodies and dendrites that contained gold-silver particles for MOR. Dually-labeled profiles were mainly dendrites that received convergent input from unlabeled axon terminals forming symmetric or asymmetric synapses. Within these dendrites, MOR often was localized within the cytoplasm, but gold-silver particles also were located along nonsynaptic portions of the plasma membrane. FG-filled proximal dendrites and perikarya contained MOR immunoreactivity that mainly was localized within the cytoplasm. These results show that MOR are located in VTA neurons that project to the mPFC, and that the primary site of MOR activation is along dendritic plasma membranes. Stimulation of MOR within these neurons would directly disinhibit mPFC target cells, and modulate excitatory and inhibitory input to these projection neurons. Supported by NIDA DA11768 to A.L.S. and DA04600 to V.M.P.

O3-12:15

CONDITONED PLACE PREFERENCE (CPP) AS A MODEL FOR RELAPSE OF DRUG USE AND THE EFFECT OF PERIPHERAL ELECTRICAL STIMULATION (PES) J.S.Han, B.Wang and F.Luo

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To date, the only animal model addressing the relapse of drug use is the extinction-reinstatement of i.v. self-administration. CPP paradigm is widely used in drug reinforcement research, yet very few studies addressed the question whether the extinguished CPP induced by morphine could be reinstated. We trained the rats with morphine (4 mg/kg) in a CPP paradigm for 10 days. 12 h after the last morphine injection, the rats were given PES of 2 Hz, 100 Hz, or 2/100 Hz, respectively for 30 min, and tested for CPP in a period of 10 min,12 h after the PES. Various kinds of control were used including restraining in the holder, foot shock, and needling without electrical stimulation. In all the control groups, the rat stayed in the drug pairing side for 8.35 – 9.02 min. The CPP score was significantly decreased in 2 Hz- and 2/100 Hz- treated groups (6.20+0.32 and 5.68+0.31 min) in a naloxone (1 mg/kg) reversible manner. 100 Hz PES was without effect. In another experiment, the established CPP was left extinguished for 9 days. The CPP can be successfully reinstated by 15 min of random foot shock stress or drug (morphine or amphetamine) priming. The results suggest that this CPP model can be used for the study of relapse of drug use (supported by NIDA grant DA 03983).

O4-18:30

ANTINOCICEPTIVE RESPONSES INDUCED BY OPIOID COMPOUNDS IN TWO DIFFERENT LINES OF MOR KNOCKOUT MICE.

R. Maldonado[1], M.A. King[2], J.M. Mitchell[1], O. Pol[3], A.G.P. Schuller[4], H. Matthes[5], G.W. Pasternak[2], B.L. Kieffer[5], J.E. Pintar[4]. (1) UPF, Barcelona (2) MSKCC, New York (3) IMIM, Barcelona (4)UMDNJ, New Jersey (5) ULP, Illkirch.

The antinociceptive responses induced by morphine-6β-glucuronide (M6G), 6-acetylmorphine and heroin have been investigated in knockout mice lacking MOR exon 1 (1) or MOR exon 2 (2) by using tail flick, tail immersion and hot plate tests. M6G (250 and 500 ng, i.c.v.) produced antinociceptive responses in both lines of MOR deficient mice, which were especially apparent in the tail-flick paradigm. In addition, M6G (5 mg/kg, i.p.) produced similar inhibition of gastrointestinal transit in both mice lacking MOR exon 1 and mice lacking MOR exon 2. Cumulative dose-responses curves of 6-acetylmorphine revealed antinociceptive effects in MOR exon 1 knockouts only when administered at the dose of 3 μg (i.c.v.), but in both lines of MOR knockouts at the dose of 9 μg (i.c.v.). Cumulative dose-responses curves of heroin (s.c) also revealed antinociceptive responses in both lines of MOR knockouts with responses in exon 1 KO significantly greater than the exon 2 KO in one of two trials. These responses were not observed after a single heroin administration. These results indicate that M6G, 6-acetylmorphine and heroin are able to produce antinociception in mice lacking exon 1 or exon 2 of MOR. The mechanisms involved in these responses remain to be clarified.

(1) Schuller et al., Nature Neuroscience 2:151-156, 1999.
(2) Matthes et al., Nature 383:819-823, 1996.

O4-18:45

PRODYNORPHIN "KNOCK-OUT" MICE DO NOT SHOW SUSTAINED NEUROPATHIC PAIN OR SPINAL OPIOID TOLERANCE.

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The consequences of injuries to peripheral nerves and opioid tolerance share common features including tactile allodynia and hyperalgesia of the hindpaws, decreased spinal opioid antinociception and upregulation of spinal dynorphin. Dynorphin may normally produce antinociception (through opioid receptors) but may be pronociceptive in pathological states (through direct or indirect actions at NMDA receptors). Here, mice with deletion of the coding region for prodynorphin (KO) or wild-type (WT) controls were studied for their sensitivity to L5/L6 spinal nerve ligation (SNL). Following SNL, both WT and KO mice showed abnormal pain within 2 days. While pain was maintained in the WT mice, the KO mice returned to baseline values by day 10 after SNL. Spinal dynorphin levels were significantly elevated at day 10, but not day 2, after SNL. Intrathecal (i.th.) MK-801 or antiserum to dynorphin blocked abnormal pain in WT animals at day 10, but only MK-801 blocked abnormal pain on day 2 suggesting an early dynorphin-independent pain and a later dynorphin-dependent pain. Repeated i.th. [D-Ala^2,NMePhe^4,Glyol^5] enkephalin (DAMGO) produced abnormal pain in WT, but not in KO, animals. The i.th. DAMGO antinociceptive dose-response curve was displaced to the right in WT but not in KO animals. DAMGO treated WT animals showed an upregulation of spinal dynorphin on day 7. DAMGO-induced tolerance and pain were reversed by either MK-801 or dynorphin antiserum. These data suggest that spinal dynorphin is required for the maintenance, but not initiation, of SNL pain. Further, dynorphin is an endogenous mediator which promotes spinal opioid tolerance, due to increased pain elicited by its NMDA receptor-dependent pronociceptive actions.

O5-11:45

REGULATION OF OPIOID RECEPTOR SIGNALING BY RECEPTOR DIMERIZATION

J.L. Whistler[#] and M. von Zastrow* UCSF Ernest Gallo Clinic and Research Center, Emeryville CA[#] and Department of Psychiatry, San Francisco CA*.

Opiate analgesia, tolerance and dependence are mediated by drug-induced activation of opioid receptors. Following activation, opioid receptors are regulated by numerous mechanisms designed to rapidly attenuate signaling. Previous studies have demonstrated that highly addictive opiate drugs such as morphine are deficient in their ability to induce the desensitization and resensitzation of opioid receptors. Specifically, morphine-activated opioid receptors elude desensitization by failing to uncouple from G protein and internalize. Recently we and others have discovered that opioid receptors can form both homo- and heterodimers. Furthermore, we have found that we can specifically affect the desensitization and resensitization properties of some of these opioid receptor dimers by manipulating their endocytic properties both pharmacologically and by utilizing mutant receptors.

O5-12:00

MOLECULAR MECHANISM OF MU OPIOID RECEPTOR DESENSITIZATION.

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Desensitization mediated by GRK and arrestin may play an important role in tolerance to opioid agonists We have characterized some of the steps in GRK3 and arrestin3 dependent desensitization of the Mu opioid receptors (MOR) using exogenous gene expression in *Xenopus* oocytes. In this system, the activation of rMOR increases the conductance of co-expressed inwardly rectifying potassium channel (Kir3.1/Kir3.4), and homologous receptor desensitization increased significantly when GRK3 and arrestin3 were co-expressed. Furthermore, care was taken in this system to ensure the absence of a receptor reserve for the responses measured. Thus this paradigm is sufficiently sensitive to measure the initial G-protein uncoupling of receptor according to the present model of GRK and arrestin mediated desensitization in which the agonist activated receptor is phosphorylated by GRK and subsequently bound by arrestin. This is in contrast to many measures of MOR desensitization in mammalian cell lines in which the intent is to monitor events down stream of uncoupling, including receptor internalization, or the presence of a large receptor reserve requires a large fraction of receptor to be uncoupled before a significant change in the physiological response can be detected. At this time point where a decrease in MOR activity can be detected, internalization is known to be occurring, blurring the distinction between receptor uncoupling and receptor internalization. Furthermore, mammalian cells are known to express a variety of GRK and arrestin homologues making it difficult to define a specific role for each species. Again this is in contrast to the *Xenopus* oocyte system where homologous desensitization of MOR requires exogenous expression of both GRK and arrestin. Here we report that removal of GRK phosphorylation sites that block internalization of MOR in cells lines do not block GRK and arrestin dependent desensitization of MOR in the *Xenopus* oocyte system. Thus our data suggest internalization of MOR in the manner well characterized in cell lines does not occurr in the *Xenopus* oocytes. In addition, these data suggest a novel site of GRK3 action for its role in uncoupling MOR from G protein. Thus, although intimately related, the determinants of internalization and desensitization of MOR may be distinct and for the reasons above difficult to distinguish in cell line studies of MOR regulation by GRK and arrestin. Our studies of MOR desensitization in *Xenopus* oocytes support this interpretation or suggest a homologue specific action of GRK3 and arrestin3 different from GRKs and/or arrestins endogenously or exogenously expressed in previous mammalian cell line studies of MOR desensitization. Supported by DA 11672

O5-12:15

CALCIUM/CALMODULIN BINDING TO MU RECEPTORS AND ITS ROLE IN OPIOID ACTIVATION OF ERK. M. Belcheva [1], M. Szûcs [1,2], D. Wang[3], W. Sadee[3] and C. Coscia[1], [1]Dept. Biochem. & Mol. Biol., St. Louis Univ. Sch. of Med., St. Louis, MO 63104; [2]Biol. Res. Center, Szeged, Hungary; [3]Dept. Biopharm. Sci. & Pharm. Chem., Univ. of California San Francisco, CA 94143.

Our recent efforts have focused on delineation of a Ca^{2+}-dependent mechanism by which opioids activate the MAP kinase phosphorylation cascade in different cell types. There is evidence to suggest a role for calmodulin (CaM) in opioid receptor signaling that entails its direct binding to the receptor. Here we report that in HEK293 cells transfected with FLAG-tagged human mu opioid receptor, DAMGO induced a time and concentration dependent activation of ERK, which appears to be modulated by CaM binding to the receptor. At nanomolar concentrations of DAMGO, a CaM-insensitive mutant mu opioid receptor (K273A) displayed a reduced ability to stimulate ERK activity compared to wild type, while at a micromolar dose of the agonist, the effect is reversed. Accordingly, CaM inhibitors, W-7 and fluphenazine, attenuated ERK stimulation by DAMGO in wild type mu opioid receptor transfected cells but failed to do so in K273A mutant cells. The data suggest a role for Ca^{2+}/CaM binding to the receptor in mu opioid modulation of ERK activity in HEK293 cells. Supported by NIDA grant DA05412.

O6-19:30

δ- AND μ-OPIOID RECEPTOR-DEFICIENT MICE EXHIBIT OPPOSING ALTERATIONS OF EMOTIONAL RESPONSES.

Brigitte L. Kieffer[1*], Dominique Filliol[1], Sandy Ghozland[2], Johanna Chluba[1], Miguel Martin[2], Hans Matthes[1], Frédéric Simonin[1], Katia Befort[1], Claire Gavériaux-Ruff[1], Andrée Dierich[3], Marianne LeMeur[3], Olga Valverde[2] and Rafael Maldonado[2]. [1] UPR 9050 CNRS, ESBS, Illkirch, France; [2] Universitat Pompeu Fabra, Barcelona, Spain; [3] Institut de Génétique et Biologie Moléculaire et Cellulaire, Illkirch, France.

The role of the opioid system in controlling pain, reward and addiction is well established, but its role in regulating other emotional responses is poorly documented by the pharmacology. Three receptors, μ- (MOR), δ- (DOR) and κ- (KOR) opioid receptors, mediate the biological activity of opioids. We have generated DOR-deficient mice and compared behavioral responses of mice lacking DOR, MOR and KOR in several models of anxiety and depression. Our data show: (i) no detectable phenotype in KOR -/-mutants, suggesting that κ-receptors do not play a major role in this aspect of opioid function; (ii) opposing phenotypes in MOR-/- and DOR-/- mutants, which contrasts with the classical notion of alike activities of μ- and δ-receptors; (iii) consistent anxiogenic- and depressive-like responses in DOR-/- mice, indicating that δ-receptor activity contributes to improve mood states.

O6-19:55

AMPHETAMINE TREATMENT AND WITHDRAWAL ALTERS LIMBIC SYSTEM NETWORK PROPERTIES.

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Administration of amphetamine produces markedly different effects on limbic system neurons depending on whether it is administered acutely or repeatedly. Acute amphetamine administration was found to alter the interaction of limbic system afferents within the nucleus accumbens, in that it tended to suppress prefrontal cortical inputs in favor of afferents from the amygdala. Upon withdrawal from repeated administration of amphetamine, a constellation of responses are induced at both cellular and network levels. Thus, there is an increase in corticoaccumbens drive, which appears to be expressed as an increase in electrotonic coupling among subsets of neurons. This occurs in concert with a down-regulation of nitric oxide synthetase in intrinsic neurons. These effects were not present while the rat was still on amphetamine, and were found to persist for up to 28 days following withdrawal. Thus, amphetamine causes an initial shift from higher cortical gating of information flow to one dependent primarily on amygdala-mediated affect. With continued administration, modulatory alterations are introduced that, upon withdrawal, produce persistent alterations in the processing of afferent inputs into this region. These results could provide an explanation for drug-induced craving, in that the resultant increased corticoaccumbens transmission would be expected to alter tonic-phasic DA balance within subcortical regions, causing the organism to take additional drug in an attempt to re-establish the steady-state.

O6-20:20

A MEMBRANE SORTING MECHANISM THAT MEDIATES DOWNREGULATION OF OPIOID RECEPTORS AFTER ENDOCYTOSIS BY CLATHRIN-COATED PITS

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Both the β- adrenergic receptor (B2AR) and δ-opioid receptor (DOR) undergo rapid, agonist-induced internalization by clathrin-coated pits. We have observed that these receptors differ significantly in their membrane trafficking after endocytosis when expressed at similar levels in HEK293 cells. B2AR recycles efficiently to the plasma membrane, either in the absence or presence of agonist, and exhibits <10% downregulation after continuous incubation of cells with agonist for 3 hours. In contrast, DOR recycles with reduced efficiency and exhibits ≥50% downregulation within 3 hours after agonist-induced activation. Downregulation observed under these conditions occurs by proteolysis of internalized receptors in lysosomes. Targeting of DOR to lysosomes is mediated by a molecular sorting operation that efficiently segregates B2AR and DOR between distinct recycling and non-recycling pathways within ~10 minutes after endocytosis, preceding the delivery of internalized receptors to lysosomes by ≥60 minutes. Multiple cytoplasmic proteins mediate this receptor sorting mechanism, including NHERF/EBP50 and ezrin/radixin/moesin -family proteins associated with the cortical actin cytoskeleton. Studies in progress seek to define additional components of the biochemical machinery mediating receptor trafficking to lysosomes. We have recently identified two integral membrane proteins present in receptor-containing endocytic vesicles, which may play an important and distinct role in mediating receptor trafficking to lysosomes.

O6-20:35

A COLLABORATIVE REPORT ON THE DEVELOPMENT OF DALDA AND [DMT[1]]DALDA. HH Szeto[1], PW Schiller[2], M Shimoyama[3], NM Lee[4], DM Desiderio[5]. [1]Weill Medical College, Cornell Univ, NY, NY, [2]Clinical Research Institute of Montreal, Canada, [3]Chiba Univ, Chiba, Japan, [4]California Pacific Medical Center, San Francisco, CA, [5]Univ of Tennessee, Memphis, TN.

DALDA (H-Tyr-D-Arg-Phe-Lys-NH_2) and [Dmt^1]DALDA (H-Dmt-D-Arg-Phe-Lys-NH_2) were designed as polar dermorphin analogs with high selectivity for the μ-opioid receptor. Biochemical characterizations of DALDA and [Dmt^1]DALDA revealed high affinity (Ki = 1.69 and 0.143 nM), extraordinary selectivity (Ki^{δ}/Ki^{μ} >10,000), and high potency (GPI; IC50 = 254 and 1.41 nM) for the μ receptor. When administered it to rats, DALDA and [Dmt^1]DALDA were 14- and 3000-fold more potent than morphine, and 2- and 4-times longer acting. In mice, [Dmt^1]DALDA was 25-50 times more potent than DAMGO it, and 50-100 times more potent than morphine sc. In mice made tolerant to morphine, ED50 for DAMGO shifted 7-fold while ED50 for [Dmt^1]DALDA increased only 2-fold. Based on its 3+ charge and limited volume of distribution (50-80 ml/kg), these data suggest that [Dmt^1]DALDA mediates peripheral analgesia. The $t_{1/2}$ of DALDA and [Dmt^1]DALDA (1.9 and 2.5 hr) are significantly longer than DAMGO (0.25 hr). Both DALDA and [Dmt^1]DALDA produced a significant increase in blood pressure after iv dose, but effect was only transient due to rapid desensitization. Even at 30 times the ED50, it [Dmt^1]DALDA did not depress respiration. These data suggest that [Dmt^1]DALDA is a superior opioid analgesic with unique pharmacokinetic and pharmacodynamic properties.

O6-20:50

REGULATION OF ADENYLYL CYCLASE ISOZYMES BY OPIATES: INSIGHT ON THE MECHANISM OF ADENYLYL CYCLASE SUPERACTIVATION

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Nine adenylyl cyclase (AC) isozymes have recently been cloned. Transfecting these isozymes into COS cells, we found that acute activation of the μ-opioid receptor inhibited AC-I, V, VI and VIII, while AC-II, IV and VII were stimulated and AC-III was not significantly affected. Chronic activation led to superactivation of AC-I, V, VI and VIII, but not of AC-II, III, IV, or VII, demonstrating that superactivation is isozyme-specific. A similar pattern of AC isozyme regulation was observed with other $G_{i/o}$-coupled receptors (δ- and κ-opioid, m_2- and m_4-muscarinic, D_2-dopaminergic, CB_1-cannabinoid), demonstrating the generality of AC superactivation. Interestingly, the weak opiates codeine/dihyrocodeine did not regulate AC activity, suggesting that their addictive properties *in vivo* could be due to their conversion to morphine/dihydromorphine. Moreover, we found that AC types V/VI interact with $G_{\beta\gamma}$ dimers, and that these dimers have a role in AC superactivation; a mutated AC-V, which was not inhibited by $G_{\beta\gamma}$, did not undergo superactivation. Moreover, chronic opiate exposure led to a change in detergent solubility of both $G_{\alpha i}$ and $G_{\beta\gamma}$ (but not of $G_{\alpha s}$), and to an increase in the phosphorylation of an ≈200 kDa molecule immunoprecipitated with an antibody to AC. These results suggest that chronic opiate regulation of AC occurs via modifications at both the AC and G protein levels. *Supported by NIDA (DA6265), the German-Israeli Foundation for Research and Development, and the Dr. Margarete Fischer-Bosch Foundation.*

O7-11:45

PHARMACOLOGICAL CHARACTERIZATION OF J-113397, A POTENT ORL1 RECEPTOR ANTAGONIST.

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J-113397 (1-[(3*R*,4*R*)-1-cyclooctylmethyl-3-hydroxymethyl-4-piperidyl]-3-ethyl-1,3-dihydro-2*H*-benzimidazol-2-one) was found to be the first potent nonpeptidyl ORL1 antagonist (Ki: cloned human ORL1 = 1.8 nM) with high selectivity over other opioid receptors. *In vitro*, J-113397 inhibited nociceptin/orphanin FQ-stimulated [^{35}S]GTPγS binding to human μ-, δ- or κ-opioid receptors up to a concentration of 100 nM, indicating selective antagonism of the antagonist on the ORL1 receptor. *In vivo*, J-113397 antagonized nociceptin (0.1 nmol, icv)-induced hyperalgesia in a dose-dependent manner in mouse/rat models of thermal pain. J-113397 showed potent analgesic activity in mouse formalin and rat carrageenin tests by itself, but had no effects on the pain responses produced by thermal and mechanical stimuli, suggesting that an ORL1 antagonist is effective in persistent pain (inflammatory and neuropathic) but not in reflex-type pain. J-113397 will be an useful tool in elucidating the physiological roles of nociceptin/orphanin FQ.

O7-12:10

REGULATORY MECHANISMS OF PROORPHANIN FQ AND PROENKEPHALIN GENE EXPRESSION IN NEURONAL AND GLIAL CELLS.

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We have studied the regulation of pOFQ gene expression in order to gain insight to the possible functions of this novel opioid-related peptide. We found that mRNA levels of the pOFQ gene are increased by injury-induced factors. Ischemic and traumatic brain injuries as well as certain neurodegenerative diseases are accompanied by elevated generation of reactive oxygen species, proinflammatory and neuropoietic cytokines. Besides having detrimental effects leading to neuronal injury and glial scar formation, these factors induce genes that are involved in repair, regeneration and neuroprotection. pOFQ gene expression was dramatically increased by oxidative stress elicited by hypoxia/ reoxygenation or hydrogen-peroxide in astroglial cells. Inflammatory mediators such as lipopolysaccharide and the cytokines IL-1β and TNFα led to an increase in pOFQ mRNA levels in the same cells. pENK mRNA levels were also increased by these treatments. In contrast, pOFQ expression was uniquely elevated by the neuropoietic cytokine CNTF in both neurons and astrocytes. We have identified the p38 and the ERK MAP kinase pathways as mediators of the regulation of pOFQ and pENK by oxidative stress. LPS increased pOFQ and pENK mRNA levels via the p38 MAP kinase cascade. The transcription factor NFκB is involved in the regulation of pOFQ but not of pENK expression by both oxidative stress and LPS. Supported by NIDA and DVHIP.

O7-12:25

A NOVEL PEPTIDE EXPRESSED IN THE MAMMALIAN BRAIN PRODUCES HYPERALGESIA AND NOCICEPTIVE BEHAVIOR IN RATS

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From total RNA isolated from mouse brain a cDNA encoding a novel secretory peptide could be amplified using the 3'- and 5'-RACE protocols. This cDNA contains an open reading frame encoding a polypeptide of 86 amino acids. Of these, the first 26 have the typical features of a signal peptide, while the following sequence of 47 residues is closely related to the peptide BV8, previously isolated from amphibian skin. Using specific primers for BV8, RT-PCR of total RNA from mouse, rat and human brain yielded a strong signal in the cerebral cortex, hippocampus, thalamus, cerebellum and spinal cord. After in situ hybridization with an antisense RNA probe, photomicrographs of brain sections confirmed the localization of the mRNA of the peptide in the pyramidal cells of the cortex, CA1, CA3 fields and dentate gyrus of hippocampus, in the cell bodies of the thalamic ventral posterolateral nucleus and of the posterior thalamic nuclear group, in Purkinje cells of cerebellum, in trigeminal nuclei and in posterior and anterior horns of spinal cord. Injection of the peptide into the left lateral ventricle of rat brain shortened the reaction time to painful stimuli (radiant heat and mechanical pressure) and induced characteristic behavioral changes, which included scream on touch (allodinia?), chattering of the teeth, front-turned vibrissa, rhinorrhea, chewing and sniffing. BV8 was also tested on one trial passive avoidance paradigm to ascertain activity on cognitive performance. Rats i.c.v. injected with BV8 20 min before training, showed a reduced latency to enter the dark compartment of the passive avoidance box 48 h later. Injection of BV8 immediately after training failed to affect the cognitive testing.

O8-10:50

GENETIC VARIABILITY OF THE HUMAN MU OPIOID RECEPTOR GENE AND ITS POTENTIAL FUNCTIONAL SIGNIFICANCE

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The human μ opioid receptor gene (*OPRM1*) is a prime candidate for substance dependence by both, biology and genetic map position. All known functionally relevant regions of this gene including 5'regulatory, exonic, and critical intronic sequences have systematically been analyzed by 'Multiplex Sequence Comparison' in 250 substance (heroin/cocaine) dependent individuals and controls. Evidence for remarkable DNA sequence variation was obtained, as demonstrated by a total of 43 variants and 52 different haplotypes predicted in the subgroup of 172 African-American substance dependent individuals and controls. These haplotypes were classified by similarity clustering into two functionally related categories, one of which was significantly more frequent in substance dependent individuals. Common to this category was a characteristic pattern of sequence variants (T-1792A, Tins-1699, A-1320G, C-111T, C17T [A6V]), which was associated with substance (heroin/cocaine) dependence. These results and their potential functional significance will be presented and discussed.

O8-11:05

ASSESSMENT OF SIGNAL TRANSDUCTION EVENTS IN LIVING CELLS USING BIOLUMINESCENCE RESONANCE ENERGY TRANSFER (BRET)

Stephane Angers, Erik Joly Dan Chelsky and Michel Bouvier Department of Biochemistry, Universite de Montreal and BioSignal Inc. Montreal, Canada.

Despite the acknowledged importance of modular assembly of proteins in signal transduction, their monitoring in living cells remains a major challenge. Here, we describe the use of BRET as an approach to study protein-protein interactions in living cells. BRET is a natural phenomenon that consists in the non-radiative transfer of energy between a bioluminescent donor (ex: *Renilla* Luciferase; *R*luc*)* and a fluorescent acceptor (ex: Green Fluorescent Protein; *GFP)*. Specific interactions can thus be monitored by constructing fusions between proteins of interest and BRET partners and, by measuring the transfer of energy that may occur as a result of their molecular proximity. In an effort to apply this technology to the study of protein interactions involved in G protein-coupled receptor signaling, co-transfection of human β2-adrenergic receptor -*R*luc (β2AR-*R*luc) and -GFP (β2AR-GFP) fusion constructs were carried out in mammalian cells. Upon addition of the membrane permeable *R*luc substrate, coelenterazine, transfer of energy was detected reflecting the existence of constitutive β2AR homodimers. Furthermore, stimulation of cells with the β-adrenergic agonist isoproterenol increased the level of BRET indicating that dimerization plays an important functional role. The agonist-dependant interaction between the regulatory protein β-arrestin and the β2AR was also monitored using a β-arrestin-GFP construct. Taken together, these data indicate that BRET is an approach applicable to the study of both constitutive and ligand-promoted interactions involved in signal transduction.

O8-11:20

RECEPTOR DOMAINS INVOLVED IN KAPPA-DELTA HETERODIMERIZATION

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Given the demonstration of kappa and delta receptor heterodimerization by Jordan & Devi (Nature 399, 697-700,1999), we have undertaken to identify the receptor domains involved in this process using chimeric opioid receptors. It was reported that, in competition studies, the heterodimerization of the kappa and delta receptors can significantly reduce the binding affinity of the individual kappa- and delta-selective ligands when the co-expressed receptors are labeled with a non-selective opioid ligand such as [3H]-Diprenorphine. We have utilized this unique pharmacological characteristic in the receptor co-expression system to screen 30 different wild type and chimeric opioid receptor combinations. Our results show that 1) There are indeed significant ligand binding property changes when the kappa and the delta receptors are transiently co-expressed in HEK 293 cells; 2) The N-terminal extracellular region, in which the kappa receptor has two Cys residues but the delta receptor has no Cys residue, is not involved in the pharmacological property change in the receptor co-expression system. 3) A region containing the fifth transmembrane domain (TM5) and the third intracelluar loop (IL3) of the kappa receptor is critical for the binding profile change seen in the receptor co-expression studies. 4) A region containing kappa IL2 and TM4 can also alter the binding profile in the receptor co-expression system, but its role is dependent on the receptor environment.

Western blotting studies using antigen-tagged chimeric receptors are underway to determine the relationship between the binding profile changes in the receptor co-expression system and the heterodimerization of the two receptor types. Together these studies can point to the structural basis of the heterodimerization process within the opioid family.

Sun01

RNA PROFILING IN A MOUSE MODEL OF SUBSTANCE ABUSE

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Inbred mouse strains differ in their response to drugs of abuse and in their propensity to voluntarily consume drugs of abuse. The C57BL/6J strain will voluntarily consume morphine to a level of ~200 mg/kg/day, leading to mortality in ~15% of the animals, while the DBA/2J strain will only consume morphine to a level of 10-20 mg/kg/day with no lethality. These strains also show differences in their responses to acute morphine injection, and in their response to chronic morphine treatment and subsequent withdrawal. We are making use of mRNA expression profiling by microarrays and by subsequent RT-PCR in C57BL/6J and DBA/2J strains (and in congenics derived from these strains) as a means of identifying pathways involved in the behavioral response to morphine. Alterations in neuronal gene expression that correlate with morphine responses can highlight pathways involved in these responses.

Sun02

RECOMBINANT HSV-1 MEDIATED TRANSFER OF PROENKEPHALIN A GENE INTO SENSORY NEURONS OF ARTHRITIC RATS - IMPROVED LOCOMOTION AND REDUCED HYPERALGESIA

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Adjuvant-induced arthritis in the rat is associated with plastic changes affecting neurons that participate in pain control, especially those that contain enkephalins. In particular, the expression of proenkephalin A (PA) mRNA becomes almost undetectable in cell bodies of sensory neurons located in dorsal root ganglia (DRG), and met-enkephalin (ME) concentrations decrease in soft tissues of ankle joints, where these neurons terminate. Studies were carried out with the aim of assessing the functional significance of decreased ME expression and of its restoration in sensory neurons by PA gene transfer in arthritic rats. Hind paws were infected with ~$2x10^6$ pfu of recombinant HSV-1 encoding rat PA (HSVE) or LacZ gene (HSVG) under the control of HSV latency associated promoter. In sham (HSVG)-infected animals (as in control arthritic rats), only scarce PA mRNA-expressing neurons were detected in lumbar DRG 3 weeks post infection (p.i.). In contrast, inoculation with HSVE resulted in a marked increase of the number of PA mRNA expressing neurons (4x) associated with a ~40% increase in ME levels in lumbar DRG. A significant reduction (-20%) of arthritis-associated thermal hyperalgesia was observed in HSVE-treated rats as compared with control or sham-infected animals. Moreover, the reduced (-85%) locomotion of arthritic rats was restored in HSVE-infected animals. Indeed, 5 weeks p.i., both horizontal mobility and rearings were markedly increased (+140 and +160%, respectively) as compared with control or sham-infected rats. Naloxone methiodide, a peripherally-acting opioid receptor antagonist, reversed these effects, suggesting that PA-derived peptides overexpressed in sensory neurons of chronically suffering rats mediated a peripheral antihyperalgesic action.

Sun03

CROSS-SENSITIZATION BETWEEN COCAINE AND MORPHINE AT THE LEVEL OF C-FOS EXPRESSION

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Repeated morphine application results not only in tolerance but also in sensitization to behavioral effects, and as found recently by us also in the expression of c-fos. After a chronic pretreatment with ascending doses of morphine (10 - 50 mg/kg over 10 days) followed by a 4 weeks drug free interval a challenge dose of morphine (50 mg/kg) caused a marked enhancement in the c-fos mRNA expression in limbic regions of the rat brain. The increase was most pronounced in the dorsomedial striatum and in the cingulate cortex. The same pattern of c-fos expression was also seen in rats which have been pretreated with cocaine (10 mg/kg twice daily for ten days) and challenged with morphine. These findings provide evidence that morphine and cocaine cause a sensitization in similar structures of the brain.

Sun04

SINGLE-STRANDED DNA BINDING COMPLEX INVOLVED IN MOUSE μ–OPIOID RECEPTOR GENE TRANSCRIPTION

J. L. Ko and H. H. Loh. Department of Pharmacology, University of Minnesota Medical School, Minneapolis, MN.

The μ-opioid receptor (mor) mediates the action of morphine in relieving pain. Previously, we reported that dual (distal and proximal) promoters are present in the mouse mor gene, with mor transcriptions in mouse brain predominantly initiated by the proximal promoter. Sp factors, bound to the double-stranded (ds) *cis*-acting elements, are critical for the proximal promoter activity. In this study, we further reported that a single-stranded (ss) *cis*-acting element and *trans*-acting protein factor are also important for mor proximal promoter activity. Mor polypyrimidine/polypurine (PPy/u) region possesses the capability of adopting ss DNA conformation. Using electrophoretic mobility shift analysis with nuclear extracts from SH-SY5Y (mor expressing cells), we demonstrated that the sense strand of PPy/u region interacts with a distinct nuclear protein, which named mor polypyrimidine binding protein (mPy). Southwestern blot analysis indicated that mPy protein is approximately 25 kDa in size. Functional assays suggested that mPy protein can *trans*-activate mor promoter as well as a heterologous promoter in SH-SY5Y cells. Moreover, combinatorial activation of both ss and ds binding factors are necessary for the proximal promoter activation. (Supported by NIDA grants)

Sun05

REGULATION OF MU OPIOID RECEPTOR GENE TRANSCRIPTION BY CYTOKINES

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A connection between the immune - and the opioid system has been shown e. g. in inflammation induced analgesia. We investigated whether cytokines can directly induce mu receptor gene transcription. SH SY5Y cells were transiently transfected with reporter gene constructs containing human and rat mu promoter sequences. Whereas IL-1 and IL-6 had no effect on reporter gene activity, 50 units/ml TNF alpha and 150 units/ml IL-4 induced transcription 2 to 3 fold. As target transcription factors for TNF alpha regulation serve typically AP-1 or/and NFkappaB. Using electrophoretic mobility shift assays (EMSA) we localized at least two binding sites for AP-1 (-2382/-1428) on the human and five (-1594/-1529/-997/-577/-358) on the rat promoter. One binding site for NFkappaB (-2166) is present on the human promoter. The involvement of these cis-elements and their cognate factors in TNF alpha regulated transcription was shown by 5' deletion analysis and EMSA-monitored cell stimulation. Similar experiments were performed to study IL-4 regulation. The mu promoters of both species each contain two putative binding sites (human -1583/-1061; rat -1353/-727) for Stat transcription factor family members, which typically mediate IL-4 induced transcription. Other cytokines including IL-1 and IL-6 may influence mu opioid receptor transcription indirectly e. g. via TNF alpha.

Sun06

HUMAN NOCICEPTIN/ORPHANIN FQ RECEPTOR GENE STRUCTURE AND POLYMORPHISM IN HEROIN ADDICTION

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Using the reported cDNA sequence we designed PCR primer pairs to probe the intron/exon organization of the human nociceptin/orphanin FQ (ORL-1) gene, which had previously been only partially mapped. We defined a 631 base intron 31 bases upstream from the first base of the methionine initiation codon. This intron had been previously predicted to exist based on alternatively spliced forms of the mRNA, as had a second intron following nucleotide position 113 of the coding sequence. Using PCR, we confirmed the existence of this second intron as well. The position of a third intron (118 bases) has been previously reported. The mapping of the ORL-1 gene organization allowed us to design PCR and sequencing primers to scan PCR amplified fragments of the gene for polymorphisms, (including single nucleotide poly-morphsms), in DNA from human subjects. We are screening a cohort of study subjects from our ongoing study of the genetics of vulnerability to addiction. Study subjects for this current study are either long-term heroin addicts currently in methadone maintenance treatment or control subjects with no history of drug or alcohol addiction/dependence. Allele frequencies of identified polymorphisms and results of association analyses between opioid dependent and control subjects will be presented.

Supported by NIH grants DA00049, DA05130 and RR00102.

Sun07

TRANSCRIPTIONAL REGULATION OF MOUSE δ-OPIOID RECEPTOR GENE.

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Three major types of opioid receptors, mu (MOR), delta (DOR) and kappa (KOR), exhibit distinct pharmacological profiles and expression patterns in the brain. It suggests that transcription regulatory elements and their associated factors are important for the distribution and density of opioid receptors. Here, we report a minimum core promoter of the mouse DOR gene, containing an E-box and a GC box that are crucial for DOR promoter activity in NS20Y cells, a DOR-expressing mouse neuronal cell line. Both functional and physical interactions between these factors were critical for the DOR promoter activity. Futhermore, the tissue specificity of the DOR gene expression has also been investigated. A DOR-nonexpressing cell line derived from mouse hepatocytes, H2.35, together with NS20Y cells were used for studing the cell-type specificity. Several reporter plasmids containing various DOR promoter regions and linker scanning mutation constructs of the DOR minimum promoter were tested in these two cell lines to test their cell-type specificity. From both *in vivo* functional assays and *in vitro* EMSA data suggested that the cell-type specificity of the DOR gene may be contributed by multiple factors. Thus the distinct developmental emergence and brain regional distribution of the DOR appear to be controlled, at least in part, by the DOR minimum promoter as well as their associated factors. (Supported by NIDA grants)

Sun08

MOR1 mRNA AND ELECTROPHYSIOLOGICAL RESPONSES TO OPIOIDS IN LC NEURONS

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Splice-variants of the cloned mu-opioid receptor have recently been identified. The purpose of the present study was to determine whether the mRNA encoding the mu-opioid receptor variant MOR1 could be identified in LC neurons responding to met-enkephalin (met-ENK) with outward currents. Slices (300um) were cut from Sprague-Dawley rats (7-21 days of age) and cells were visualized using IR-DIC optics. Following whole-cell patch recording, the cell contents were partially aspirated and subjected to reverse transcriptase-polymerase chain reaction (RT-PCR). Primers specific for MOR1 were used to characterize the recorded neurons and the identity of amplicons was tested by Southern analysis. LC neurons responded to 1.0 uM met-ENK with currents of 43-245 pA and the majority of met-ENK responsive neurons were positive for MOR1 mRNA. Control experiments suggested that the presence of the MOR1 sequence was due neither to contamination by mRNA from damaged cells nor to carry-over contamination of the PCR. Thus, MOR1 could mediate responses to opioids in at least some LC neurons. Supported by PHS grants DA09642 and DA05466 from NIDA.

Sun09

A DELTA OPIOID RECEPTOR LACKING THE 3rd CYTOPLASMIC LOOP IS GENERATED IN HUMAN MALIGNOMAS

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RT-PCR and radioligand binding revealed the presence of delta opioid receptors in human melanoma cell lines. An additional, shorter amplificate was also detected which was due to a deletion of 144 base pairs (bp) within the third exon. The excised fragment corresponded to the third cytoplasmic domain of the receptor protein. The gene sequence displayed no abnormalities; therefore this short product resulted from mRNA processing. The 144 bp deletion was not detected in normal human melanocytes nor in human or rat brain. However, it was present in SH-SY5Y human neuroblastoma cells. Therefore, its appearance tightly correlated with malignancy. Transfection experiments indicated a spontaneous biological activity of the altered delta opioid receptor which could be important for tumor genesis.

Sun10

QUANTITATIVE TRAIT LOCI (QTL) ANALYSIS OF MORPHINE-INDUCED ANTINOCICEPTION IN THE SMXA RECOMBINANT INBRED STRAINS OF MICE.

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Most drug responses reflect both genetic and environmental influences. The genetic influence on drug responses can be determined using inbred strains or recombinant inbred (RI) strains of mice. The present study was then designed to investigate the determination of the chromosomes and loci associated with the morphine-induced antinociception using A/J, SM/J and their recombinant inbred (SMXA RI) strains of mice. Antinociception was measured using the tail-flick method. In A/J mice, morphine (5 mg/kg, s.c.) produced a significant antinociception, whereas morphine induced a weak antinociception in SM/J mice. In SMXA RI strains, morphine-induced antinociception was found to be distributed unimodally.Quantitative trait loci (QTL) analysis of these data indicate that the morphine-induced antinociception is mediated by 20 loci on chromosomes 2, 4, 9, 10, 15 and X. The chromosomes identified by this study include Htr1d, Gad1 and Drd2 gene. These genes code for 5-HT_{1B} receptor, glutamic acid decarboxylase and dopamine D_2 receptor, respectively. Thus, the present data suggest the possibility that these factors may be dominantly implicated in the regulation of morphine-induced antinociception.

Sun11

NALOXONE TREATMENT OF ADULT HIPPOCAMPAL PROGENITORS

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Opioids and their receptors have been implicated in the progress of development in different in vitro and in vivo studies. The aim of this study was to elucidate the effect of naloxone on adult hippocampal progenitors from rat. Treatment with naloxone (100 uM) for two days changed the mRNA levels (> 2 –fold) for several genes involved in cell cycling as diplayed by DNA-array. In accordance with this, incorporation of [^{3}H]thymidine after the same treatment decreased the proliferation significantly. Cultures exposed to naloxone (100 and 10 uM) for ten days showed that many cells became positive for neuron markers. Further characterisation will show the nature of the differentiated progenitors and whether they might be of potential therapeutic use.

Sun12

DELTA OPIOID RECEPTOR GENE: TRANSCRIPTIONAL REGULATION.

D. Smirnov, H.J. Im, and H.H. Loh. Department of Pharmacology, University of Minnesota Medical School, Minneapolis, MN.

In the past, cell cycle-dependent modulation of the mouse delta opioid receptor (DOR) protein and changes in the receptor properties were reported. In our present work, we analyzed transcriptional regulation of the DOR gene by using NG108-15 cells that were synchronized in different phases of the cell cycle. Protein-DNA interactions in the DOR gene basal promoter were identified using DMS *in vivo* footprinting analysis. We found one putative Sp1 site protected from DMS methylation during the G_0/G_1 phase. In addition, partial protection was shown in the G_0/G_1 cells of the region defined as X-Not1. During all phases of the cell cycle partial protection of E-box and hypersensitivity on the borders of this *cis*-element occurred in the NG108-15 cells. All these protein-DNA interactions were present in cells endogenously expressing DOR, which were the NG108-15 hybrid cells, but not in the N2A cells. The importance of Sp1 and E-box sites were shown by mutant construct analysis. Consistent with the footprinting result, the maximum DOR mRNA level was detected in NG108-15 cells that accumulated in the G_0/G_1 phase. Furthermore, run-off assay confirmed the active DOR transcription in this phase. Our data demonstrate that the cell cycle-dependent up-regulation of the DOR gene in NG108-15 cells is mediated by specific protein-DNA interactions. (This work is supported by NIDA grants.)

Sun13

MOUSE MU-OPIOID RECEPTOR DISTAL PROMOTER TRANSCRIPTIONAL REGULATION BY SOX PROTEINS

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Mouse mu-opioid receptor (MOR) gene has two promoters, proximal promoter and distal promoter. In adult mouse brain, the activity of proximal promoter verses distal promoter is approximately 20:1. A 34-base pair DNA fragment, called silencer, positioned between the two promoters is responsible for the blockade of MOR gene expression from the distal promoter. To understand the underlying mechanisms of regulation of the distal promoter, we report here the isolation and characterization of mSOX18 and mSOX21, which are from sox gene family associated with developmental pathway. Using yeast one-hybrid system with a 40-base pair DNA fragment around silencer as cis-acting regulatory element, we screened the adult mouse cDNA library and isolated five cDNA clones, two of them encoding a polypeptide with significant sequence homolog to human hSOX21 and being designated mSOX21. Another two are mSOX18 and the other is mSOXLZ. By transient expression analysis, we demonstrated that mSOX18 was able to up-regulate and mSOX21 down-regulate distal promoter activity in both CHO and NMB cells. Further deletion analysis suggested that their DNA-binding site is about 15 nucleotides upstream of the silencer, consistent with the putative binding site of sox family proteins. In conclusion, different sox proteins are able to either up-regulate or down-regulate the MOR distal promoter activity. Whether the distal promoter plays a role in MOR expression in the mouse developmental stages needs to be further investigated.

Sun14

SINGLE NUCLEOTIDE POLYMORPHISM OF THE HUMAN KAPPA OPIOID RECEPTOR.

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The kappa opioid receptor (KOR) and its natural ligand dynorphin are important mediators of the basal and drug-induced activity of dopaminergic neurons. We hypothesize that genetic variability in the human KOR may alter the function or expression of the receptor and may contribute to individual variability in vulnerability to opiate and possibly other addictions. From a cohort of 450 subjects entering a study of the genetic basis of the addiction, we have identified the following two groups: a) a control group of individuals with no history of drug or alcohol abuse or dependence, and (b) subjects with long-term opiate addiction, with or without codependence on cocaine, alcohol, or other drugs. Genomic DNA was isolated from blood specimens from each subject. PCR amplified DNA from the coding region of exon 3 of the KOR gene was sequenced. Sequencing electrophoregrams were evaluated for single nucleotide polymorphism (SNP) in 150 subjects. Analysis of the sequences has revealed three SNPs in exon 3: two high frequency SNPs in TM VI domain and a low frequency SNP in the C-terminal area. These SNPs do not change predicted amino acids sequence. Variations in SNP frequency among study groups will be compared and statistical analysis of these variations will be presented. Supported by NIH grants DA05130, DA00049, and RR00102

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IMMUNOHISTOCHEMICAL LOCALIZATION OF A CARBOXY TERMINUS EPITOPE OF THE NOVEL MU OPIOID RECEPTOR SPLICE VARIANT MOR-1C WITHIN THE HUMAN SPINAL CORD

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The present study examined immunohistochemically the distribution in the human spinal cord of a splice variant of the mu opioid receptor, MOR-1C, using a rabbit antiserum generated against a unique epitope from this new carboxy terminus sequence. MOR-1C-like immunoreactivity (-LI) was particularly abundant in the superficial laminae of the dorsal horn and around the central canal. In the substantia gelatinosa, MOR-1C-LI appeared in small diameter axonal elements as well as in the cytoplasm and plasmalemma of small spinal neurons and dendrites in inner lamina II. Some MOR-1C-LI fibers were also observed in the Lissauer's tract. Comparatively in the human cord, mu opioid receptor-LI (MOR-1-LI) and delta opioid receptor-LI (DOR-1-LI) were only observed in the superficial laminae. MOR-1-LI appeared to be diffuse whereas MOR-1C-LI and DOR-1-LI were in thin beaded processes that are probably axonal. We showed that opioid receptors distribution differed in the spinal cord where they might play a role in the control of pain processing.

Sun16

DOPAMINERGIC NEUROTRANSMISSION IN THE NUCLEUS ACCUMBENS OF KAPPA-OPIOID RECEPTOR (KOR) KNOCKOUT MICE: AN IN VIVO MICRODIALYSIS STUDY.

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There is evidence of an interaction of κ-opioid and DA systems. Thus, presynaptic κ-receptors have an inhibitory effect on the DA release in the n.accumbens (NAcc). However, mechanism underlying this interaction and its implications for drugs of abuse remain relatively unknown.

In this study conventional and no net flux in vivo microdialysis were used to ascertain basal and cocaine-evoked levels of extracellular DA and the rate of DA uptake in NAcc of male KOR knockout, heterozygous and wildtype mice. Conventional microdialysis revealed that basal as well as cocaine-evoked dialysate levels of DA in NAcc were significantly higher in KOR mice as compared to wildtype controls. No net flux studies revealed no difference in basal extracellular DA between genotypes. However, the extraction fraction of DA (an indirect measure of the rate of DA uptake) was greater in KOR animals as compared to wildtype controls. Because the extracellular levels of DA are determined by both its release and uptake, the fact, that the extracellular DA levels were not different, whereas the rate of DA uptake was higher in KOR knokout mice, attest that these animals have greater DA release as compared to wildtype controls. Therefore, one can assume that in the absence of inhibitory control through κ-opioid receptors in mutants DA release in NAcc is increased and this results in a compensatory increase in DA uptake.

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COEXPRESSION OF IMMUNOREACTIVITY FOR DOR1 AND KOR1 IN SPINAL DORSAL HORN.

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Previous studies have reported that δ- and κ-opioid receptors can exist as dimers either with themselves (e.g., δ-δ) or with each other (i.e. κ-δ). In the present study we examined whether single structures expressing immunoreactivity for both the cloned δ- and κ-opioid receptors (DOR1 and KOR1 respectively) could be observed in the spinal cords of rats.

Cryostat sections of rat spinal cord were cut and immunofluorescently stained for DOR1 and KOR1. The primary antisera were raised in rat (DOR1) or rabbit (KOR1); secondary antisera were labeled with Cy2, Cy3 or Cy5. Sections were examined using confocal fluorescence microscopy.

Varicose fibers or individual varicosities that appeared to be double-labeled for DOR1 and KOR1 were observed in the spinal superficial dorsal horn. Control experiments suggested that the double-labeling was not artifactual.

We conclude that co-expression of DOR1 with KOR1 may occur in the superficial dorsal horn, and that DOR1/KOR1 dimerization might play a role in the actions of opiates in this region. Supported by PHS grant DA05466 from NIDA.

Sun18

LACK OF LONG-TERM POTENTIATION IN THE DENTATE GYRUS OF μ OPIOID RECEPTOR-DEFICIENT MICE

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Tetanic stimulation of the lateral perforant pathway in transversal hippocampal slices of wild-typ mice causes a long-lasting potentiation (LTP) of the field potential recorded in the dentate gyrus. This potentiation, however, was absent in the dentate gyrus of μ opioid receptor (MOP) deficient mice. In contrast, the lack of MOP in these animals did not affect LTP in the CA1 region of the hippocampus after tetanic stimulation of the Schaffer collaterals. In wild-type mice the specific MOP antagonist funaltrexamine decreased LTP in the dentate gyrus but not in the CA1 region. The lack of MOP binding in the hippocampus of MOP deficient mice was accompanied by a reduction in D2 binding sites, whereas D1 and glutamate binding were not changed. These observations are in line with earlier observations in rats and demonstrate an essential role of MOP activation for LTP induction in the dentate gyrus but not in the CA1 region.

Sun19

OPIOID AND $GABA_A$ RECEPTORS ARE CO-LOCALIZED IN RAT BRAIN.

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Previous studies have reported that some cells that are indirectly disinhibited by mu-opioid agonists are also directly inhibited by kappa-opioid agonists. However, it's not clear whether a single neuron may interact with both opioids and GABA. In the present study, we investigated whether neurons in rat midbrain and medulla express both opioid and $GABA_A$ receptors. Coronal sections through rat brain were double-stained using antibodies against the alpha 1 subunit of $GABA_A$ receptor that were combined with antibodies either against the cloned mu-opioid receptor (MOR1) or the cloned kappa-opioid receptor (KOR1). Neurons double-labeled for $GABA_A$ receptors and either MOR1 or KOR1 were found in many brain regions including PAG, inferior colliculus, mesencephalic trigeminal nuclei, pontine reticular nuclei and nucleus raphe magnus. Neurons double-labeled for $GABA_A$ and MOR1 were observed less frequently than those labeled for $GABA_A$ and KOR1. Our findings provide anatomical evidence that GABAergic and opioidergic systems are closely linked and activity of the same neuron may be regulated directly by both GABA and opioids. In addition, our findings provide anatomical evidence that opioid agonists may both indirectly disinhibit, and directly inhibit, the same cells. The latter suggests that the actions of opioids are tightly constrained by the circuitry upon which they act. *Supported by PHS grant 1KO1 DA000391-01A1 from the National Institute on Drug Abuse to Alexander E. Kalyuzhny*

Sun20

ETHANOL INDUCTION OF FOS IMMUNOREACTIVITY IN MU OPIATE RECEPTOR KNOCKOUT MICE

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Using immunocytochemical techniques, we have examined FOS immunoreactivity (FOSir) induced by either i.p. injection of ethanol (20 % w/v) or saline in both wild-type (WT) and mu opiate receptor knockout (KO) mice. For the saline-treated animals, modest to strong FOSir was detected in some brain areas of KO mice including paraventricular thalamic nuclei (PVA/PV/PVP), dorsal hypothalamic areas (DA) and supramammillary nuclei (SuM) and sparse FOSir in the hypothalamic paraventricular nucleus (PVN) and anteroventral preoptic nucleus (AVPO). No obvious FOSir was detected in these areas in WT mice. For the ethanol-treated animals, FOSir was detected in many nuclei in both WT and KO mice including the PVN, Edinger-Westphal nucleus (EW), parabrachial nucleus (PB), locus coeruleus (LC) and supraoptic nucleus (SON) that were reported previously using a rat model (Chang et al 1995). However, no clear FOSir was seen in the bed nucleus (BNST) and central amygdala (Ce) in either WT and KO mice, although ethanol was reported to clearly induce FOSir in these areas in the rat brain. No further induction of FOSir by ethanol was seen in the PVA/PV/PVP, DA and SuM in the KO mice, while ethanol did induce strong FOSir in these areas in the WT mice. Overall, the net intensity of FOSir was similar in these areas in both WT and KO mice. Ethanol induced modest FOSir in the ventral lateral genicular nucleus (VLG), periventricular hypothalamic nucleus (Pe), and suprachiasmic nucleus (Sch) in WT mice, but not in KO mice. In contrast, no evident FOSir was induced by ethanol in the septohypothalamic nuclei (SHy) and anterior olfactory nuclei (AOP) in the WT mice, however modest FOSir was clearly seen in these areas in the KO mice. Taken together, mu opiate knockout mice seems to have elevated basal FOSir and altered ethanol induction of FOSir in some specific brain areas.

Sun21

DYNORPHIN B AND ENDOMORPHIN-1 INHIBIT THE ACETYLCHOLINE EVOKED CURRENTS IN HAIR CELLS.

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The co-existence of the opioid peptides with acetylcholine (ACh) in the inner ear suggests their modulatory role in the regulation of efferent cholinergic transmission. We studied the effects of dynorphin B and endomorphin-1 on the ACh-evoked currents in patch-clamped isolated frog saccular hair cells. Here we show that both, mu- (endomorphin-1) and kappa- (dynorphin B) opioid receptor agonists, dose-dependently and reversibly inhibit the ACh produced response. The effect of dynorphin B was only partially antagonized by naloxone and nor-Binaltorphimine. The effect of endomorphin-1 was partially antagonized by naloxone but was not sensitive to either diprenorphine or CTOP. Previously we have demonstrated that morphine can directly interact with alpha9 nicotinic receptors in hair cells and Xenopus oocytes. However, the possible involvement of the opioid receptors in the interaction of opioids with ACh receptors could not be ruled out. Full length cDNA's for opioid receptors have not been cloned from frog, however 162 bp PCR fragments of mu- (J Molec Evol 43:179-,1996), delta- and kappa receptors have been cloned from Bull frogs. Using aRNA methodology to amplify mRNA from single cells, followed by hybridization to cDNA clones of opioid receptors, as well as the frog PCR fragments, we found that hair cells express all three opioid receptor mRNAs. The effects of endogenous opioids in the hair cells could therefore be mediated in part by opioid receptors and by direct actions at ACh receptors.

Sun22

OPIATE INDUCED MESOLIMBIC DOPAMINE RELEASE AND LOCOMOTION IN INBRED MICE.

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Extracellular dopamine (DA) levels in the nucleus accumbens (NucAc) of freely moving mice were sampled by microdialysis during morphine (3mg/kg subcutaneous) administration. As previously reported, morphine induced increases in locomotion in C57BL and Sv129 mice with little locomotor activation in DBA2 mice. Basal NucAc DA levels were similar in C57BL and 129Sv mice though were approximately 25% lower in DBA2 mice. Morphine induced the most pronounced increase in extracellular DA in C57BL mice (approximately 60%). Smaller increases in extracellular DA levels (approximately 30%) were noted in Sv129 and DBA2 mice. Administration of heroin (3mg/kg subcutaneous) the following day induced a similar (approximately 80%) increase in mesolimbic DA levels in all animals whereas the locomotor activation followed the same rank order of magnitude as seen after morphine administration though magnified several fold. No appreciable degree of sensitization was evident in any of the strains studied.

Thus, morphine's ability to activate the mesolimbic system bears little relationship to its locomotory effect. Furthermore, opiate induced activation of the mesolimbic system reaches a maximum effect long before a ceiling is reached for locomotion.

Sun23

DIFFERENTIAL TARGETING OF CB1 AND μ-OPIOID RECEPTORS IN THE RAT STRIATAL PATCH.

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The motor and physiological effects of cannabinoids reflect activation of cannabinoid subtype 1 (CB1) receptors that are present in many brain regions, including the caudate-putamen nucleus (CPN). In this region μ opioid receptors (μOR) are enriched in patch compartments and also play a role in motor function. We used electron microscopic immunocytochemistry to determine the subcellular localization of CB1 in CPN patches of the rat brain that were identified by immunolabeling for μOR. CB1 receptor labeling was mainly seen in spiny dendrites and spiny as well as isolated aspiny-type somata. In dendrites, CB1 was mainly localized to plasma membranes and cytoplasmic organelles. These sites were distinct from μOR, which were also primarely seen in spiny dendrites, a few of which contained both receptors. Axons and axon terminals as well as abundant glial processes also showed plasmalemmal CB1. The CB1-labeled glial processes were often apposed to excitatory type axospinous synapses. Our results suggest that CB1 receptors and μOR receptors differentially modulate the output of largely separate populations of spiny neurons residing in the CPN patch. In addition, CB1 receptors may modulate neuronal activity indirectly through action on perisynaptic glia within this region. Support: NIDA DA04600 to V.M.P.; DA00256/11322 to K.M..

Sun24

FREQUENT COLOCALIZATION OF THE μ-OPIOID RECEPTOR AND CaMKII IN PAIN-PROCESSING BRAIN REGIONS

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The μ-opioid receptor (MOR1) mediates the main analgesic effects of morphine and several other opioids. However, the clinical benefit of these drugs is limited by the development of tolerance and dependence. *In vitro* the μ-opioid receptor undergoes a rapid homologous desensitization during prolonged agonist exposure. We have recently identified the serine residues, Ser^{261} and Ser^{266}, within the third intracellular loop as two consensus calcium/calmodulin-dependent protein kinase II (CaMKII) sites required for agonist-induced phosphorylation and desensitization of the μ-opioid receptor in HEK 293 cells. Since the specific pattern of μ-opioid receptor regulation *in vivo* is thought to depend on the cell- and tissue-specific complement of protein kinases, we examined the spatial relation between MOR1 and CaMKII in rat brain using specific antibodies. We found that MOR1 and CaMKIIα which is a major CaMKII isoform expressed in the central nervous system show overlapping distributions in many pain-processing brain regions including the superficial layers of the spinal cord dorsal horn and dorsal root ganglia. At high power magnification it was evident that virtually all MOR1-expressing nociceptive spinal cord neurons also co-contain CaMKII. In naive or saline-treated animals the μ-opioid receptor was almost exclusively confined to the plasma membrane, while CaMKII was localized to vesicle-like structures throughout the cytoplasm. After subcutaneous administration of the μ-opioid receptor agonist, etorphine, a large proportion of the μ-opioid receptor proteins redistributed from the plasma membrane into the cytosol where it was frequently co-localized with CaMKII. Together, we identify CaMKII as a potential protein kinase, which by virtue of its colocalization with MOR1 may be in a position to phosphorylate the μ-opioid receptor and may thus contribute to the development of tolerance to opioid analgesics.

Sun25

CELLULAR LOCALIZATION OF OPIOID RECEPTORS IN ASTROCYTES AND OLIGODENDROCYTES IN NEWBORN MOUSE BRAIN.

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There is abundant evidence that all three opioid receptors (μOR, κOR, δOR) are expressed in newborn rodent CNS, but there are few reports examining the patterns of OR expression in developing glia *in vivo*. Using brains from postnatal day 2-5 ICR mice, μ, δ, and κ OR were co-localized in astrocytes (GFAP) and oligodendrocytes (O4) in three regions of the developing brain: ventricular lining cells (V), subventricular zone (SVZ) and striatum (S). Anti-NeuN was used to identify neurons. μOR immunoreactivity was most abundant in V (about _ of cells) with none present in GFAP or O4 positive cells. μOR positive cells decreased to $1/5^{th}$ of cells in the SVZ and S, with $1/3^{rd}$ of those being co-localized in both glial types. κOR positive cells were absent in V but increased to $1/10^{th}$ of cells in S. A small percent of κOR positive cells co-localized with GFAP in SVZ, but in striatum $1/3^{rd}$ of κOR positive cells also exhibited GFAP and another $1/3^{rd}$ were O4 positive. δOR was virtually absent from V and SVZ and less than a tenth of cells in S were positive. Co-localization of δOR and GFAP or O4 was minimal. These findings support the hypothesis that subpopulations of astroglia and oligodendroglia exhibit both μ and κ opioid receptor immunoreactivity in the developing brain. In the progression from lining cells to striatum, there appears to be a decreasing gradient of μOR and an increasing gradient of κOR reactivity. Supported by NIDA (DA 06204).

Sun26

SEX DIFFERENCES IN DELTA OPIOID RECEPTOR IMMUNOREACTIVITY IN AMYGDALA.

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Although gonadal hormones can modulate opioid systems, the demonstration of sex differences in specific opioid systems is less consistent. This study compared enkephalin-like immunoreactivity (ENKir) and delta opioid receptor immunoreactivity (DORir) in the amygdala of male and cycling female rats (n=3 each). Sections from male and female brains were simultaneously processed for ABC peroxidase immunohistochemistry. DORir was found only in axon terminals and in most amygdalar nuclei, but was particularly dense in the central nucleus and posterodorsal subdivision of the medial nucleus (Mpd). In the medial nucleus most DOR-positive axon terminals were scattered throughout the neuropil. In all three male-female pairs, the density of DOR-positive axon terminals in the medial amygdalar nucleus was higher in the male than in the female. This difference was seen in all four subdivisions of the nucleus but was most pronounced in the Mpd.

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Sun27

EXPRESSION OF BIOLOGICALLY ACTIVE β-ENDORPHIN IN K562 CELLS

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The aim of this study was to examine different cDNA constructs of the rat POMC-gene that were transiently transfected into K562 cells for their expression of ß-endorphin (END) and their functional relevance in pain inhibition. Therefore, we constructed the following vectors: pMOIK1-POMC containing the full rat POMC-cDNA under the control of a CMV-Promoter (P_{CMV}), pMOK-POMC containing the full rat POMC-cDNA under the control of P_{CMV} with an intronic enhancer sequence, pMOK-END containing only the END-cDNA sequence under the control of a CMV-Promoter, and the control vectors (pMOK, pMOIK1) without any POMC-cDNA sequence. Determination of END peptide by a specific radioimmunoassay showed that the pMOK-POMC vector expressed END up to 4 ng/10^6 cells, whereas the pMOIK1-POMC and pMOK-END expressed END poorly (1 ng/10^6 cells and 100 pg/ 10^6 cells, respectively). As expected, in K562 cells transfected with the control vectors END was not detectable. Biological activity of the recombinant END was assessed by algesiometry. Four days after injection of Freunds complete adjuvant into the hind paw of male Wistar rats, antinociceptive effects of i.pl. injection of lysates of transfected K562 cells were measured by paw pressure algesiometry. Administration of pMOK-POMC lysate (volume 200 µl, corresponding to 1,6 x 10^6 lysed cells) that contained recombinant END resulted in a significant antinociceptive effect compared to the lysate of control vector (pMOK) (14.51 ± 1.29 PPT versus 0.52 ± 0.86 PPT, $P<0.05$, Dunnett). Thus, successful expression of biologically active END in K562 cells appears to depend on the full length POMC sequence and an inserted intronic enhancer sequence near the CMV promoter.

Sun28

SEX DIFFERENCES IN KAPPA OPIOID ANTINOCICEPTION

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The present study was conducted to determine the generality of sex differences observed in mu opioid antinociception by examining seven opioid agonists that varied in selectivity and efficacy at kappa versus mu receptors. Complete dose- and time-effect curves were obtained for U69,593, U50,488, (-)-bremazocine, (-)-pentazocine, ethylketazocine, butorphanol and nalbuphine on the 50°C and 54°C hotplate and warm water tail withdrawal assays in the rat; locomotor activity was also measured 32-52 min post-injection. On the 50°C hotplate, nalbuphine produced significantly greater antinociception in males than females at some doses; additionally, males showed greater antinociception than females at 0.01 mg/kg and females showed greater antinociception than males at 0.03 mg/kg (-)-bremazocine. There were no sex differences in the antinociceptive effects of any agonists on the 54°C hotplate. On the 50°C tail withdrawal assay, all opioid agonists produced significantly greater antinociception in males than females at one or more doses; however, on the 54°C tail withdrawal assay, only (-)-pentazocine and (-)-bremazocine produced significantly greater antinociception in males than females at one or more doses. All agonists decreased spontaneous locomotor activity with no consistent sex differences. The greater sensitivity of the tail withdrawal assay to sex differences in kappa opioid antinociception suggests that these differences may be more spinally than supraspinally mediated. (Supported by NIDA Grant DA10284)

Sun29

CLONIDINE-INDUCED ANTINOCICEPTION IS REDUCED BY DEXAMETHASONE IN MICE

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The effects of dexamethasone pretreatment on clonidine-induced antinociception was investigated in mice. In the hot plate and the tail flick tests, dexamethasone administered intraperitoneally at a dose of 1 mg/kg, 15 min before clonidine did not change clonidine antinociception, whereas administered 30 or 60 min before clonidine it reduced clonidine antinociception in both tests. A low dexamethasone dose (0.1 mg/kg) administered 30 min before clonidine was not able to change clonidine-induced effect, while a higher dexamethasone dose (10 mg/kg) induced the same effect observed at the dose of 1 mg/kg in the hot plate and in the tail flick test. Cycloheximide administered at the dose of 10 mg/kg 2 h before clonidine did not change clonidine-induced effects in the hot plate test and in the tail flick test, but was able to prevent dexamethasone effects on clonidine-induced antinociception. The glucocorticoid receptor antagonist RU38486 administered centrally at the dose of 1 ng did not change clonidine-induced effects, whereas it was able to block dexamethasone effects on clonidine-induced antinociception. These results suggest that the dexamethasone effects on clonidine-induced antinociception depend on the stimulating effects that dexamethasone exert on the protein synthesis via the glucocorticoid receptor in the brain.

Sun30

WHICH TYPES OF OPIOID RECEPTORS ARE INVOLVED IN THE ANTINOCICEPTIVE EFFECTS OF RB101(S)?

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We have previously shown that RB101, a complete inhibitor of enkephalin-degrading enzymes, decreased carrageenin-induced c-Fos protein expression at the spinal cord level. We now investigated the respective role of the three main types of opioid receptors (μ, ∂, or k) in the depressive effects of the enantiomer RB101(S) in these inflammatory conditions. We used specific opioid receptor antagonists: ß-funaltrexamine (FNA), naltrindole (NTI) and nor-binaltorphimine (BNI), as nonpeptide naltrexone derived antagonists for μ, ∂ and k opioid receptors, respectively. RB101(S) (30 mg/kg, iv) was injected 10 min before intraplantar injection of carrageenin (6 mg/150 μl of saline) in awake rats. FNA (10 mg/kg, iv) was injected 24 h before carrageenin. NTI (1 mg/kg, iv) and BNI (2.5 mg/kg, iv) were injected simultaneously with RB101(S), and a second injection was done 40 min later. Control rats received respective vehicle injections. C-Fos-protein immunoreactivity (FIR) was evaluated in the lumbar spinal cord 90 min after carrageenin. FIR neurons were preferentially located in the superficial (I-II) and deep (V-VI) laminae of segments L4-L5 (areas containing numerous neurons responding exclusively, or not, to nociceptive stimuli). RB101(S) significantly reduced the total number of FIR neurons (30% of reduction, $p<0.01$). This effect was completely blocked by FNA and NTI. In contrast, BNI did not reverse the effects of RB101(S). These results suggest that functional interaction occurs between μ and ∂ opioid receptors in enkephalin-induced antinociceptive effects. This cross-talk may involve opioid receptors located on separate neurons or arise from receptor complex. Supported by ARC #9605 and MILDT #98D11.

Sun31

THE MOR PARTICIPATES IN DPDPE,DELT II-MEDIATED SPINAL ANTINOCICEPTION BUT IS NOT REQUIRED FOR DOR-α_2 ADRENERGIC SYNERGY¶.

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The contribution of the μ opioid receptor (MOR), in δ opioid receptor (DOR) agonist-mediated antinociception has been previously studied but remains to be fully characterized. We compared the effects of intrathecal administration (i.t.) of the DAMGO, morphine; the DOR agonists DPDPE and deltorphin II (DELT II) and the $\alpha_2$adrenergic receptor agonist, UK-14,304, in MOR-knockout (MOR-KO) and wildtype (WT) mice in the substance P nociceptive test. Antinociception induced by i.t. both morphine and DAMGO was ablated in MOR-KO mice. However, the potency of an $\alpha_2$adrenergic receptor-selective agonist UK-14,304 was not altered in MOR-KO mice. In contrast, the potency of i.t. DELT II and DPDPE decreased 16- and 250-fold, respectively in MOR-KO mice. The DOR-selective antagonist, naltrindole, reversed DELT II- and DPDPE-mediated antinociception in MOR-KO, but not in WT mice. Therefore, DELT II- and DPDPE- mediated spinal antinociception may be governed by MOR and DOR at low and high doses, respectively. Interestingly, isobolographic analysis showed that, despite substantial loss of DPDPE potency in MOR-KO mice, DPDPE-UK-14,304 antinociceptive synergism is fully retained. Collectively, these experiments demonstrate that 1) MOR participates in DELT II- and DPDPE-mediated spinal antinociception and 2) the presence of MOR is not necessary for DOR-α_2 adrenergic receptor analgesic synergy.

Sun32

VERY LOW DOSE NALTREXONE ENHANCES MORPHINE ANALGESIA IN HUMANS

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Pre-clinical studies show that very low (pM) concentrations of opioid antagonists selectively antagonize excitatory but not inhibitory opioid functions in vitro and in vivo and increase morphine's analgesic potency. We conducted a 120 patient randomized, double blind, placebo controlled, single dose post-operative dental study. Patients received 60mg oral morphine or the same morphine dose in combination with one of three low doses (1.0, 0.1, 0.01mg) of oral naltrexone, an opioid antagonist. All treatment groups reported significantly greater analgesia than placebo. Patients receiving the two lower doses of naltrexone (0.1 and 0.01mg) combined with morphine reported greater analgesia than did morphine alone. The lowest dose naltrexone combination was the most potent, resulting in ~30% greater analgesia than morphine alone (PID at hrs. 1-3, $p<0.05$). The addition of very low doses of naltrexone significantly enhances the analgesic potency of morphine in patients following dental surgery.

Sun33

ENDOMORPHIN-2-LIKE IMMUNOREACTIVITY IN THE MOUSE SPINAL CORD DECREASES AFTER CHRONIC CONSTRICTION INJURY.

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Endomorphin 2 (Tyr-Pro-Phe-Phe-NH_2, EM2) is an endogenous morphine-like substance that binds to the mu-opioid receptor with high affinity and selectivity. Previous studies have demonstrated EM-2-like immunoreactivity (EM-2-LI) in the superficial layers of the dorsal horn in the spinal cord and in primary afferents, suggesting a role for this peptide in pain transmission. To determine whether EM-2-LI is altered in the spinal cord of mice after nerve injury, the left sciatic nerve of Swiss Webster and ICR mice was exposed and ligated with 4-0 chromic gut. In control animals, the sciatic nerve was exposed but not ligated. Two weeks after ligation, changes in EM-2-LI were assessed by immunocytochemistry according to our standard protocol. In mice with sciatic nerve ligations, the side of the spinal cord ipsilateral to the nerve injury exhibited less EM-2-LI relative to the contralateral side and to control animals. The change was restricted to the medial dorsal horn in the lumbar segments innervated by the sciatic nerve. Thermal hyperalgesia was apparent after surgery as evidenced by significantly decreased paw withdrawal latencies from 4 to 14 days after nerve injury. The decrease in EM-2-LI during the development of chronic pain is consistent with the loss of an inhibitory influence on pain transmission, and suggests that EM-2-LI may be modulated in this nerve injury model of chronic pain.

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ANTI-ARTHRITIC EFFECTS OF THE DELTA-SELECTIVE OPIOID ANTAGONIST HS 378

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Currently available therapies for rheumatoid arthritis are unsatisfactory and include non-steroidal anti-inflammatory agents and disease-modifying anti-rheumatic drugs. Recently, it has been described that delta-selective opioid antagonists exhibit immunosuppressive properties. The present study was designed to investigate the therapeutic effect of the delta-opioid antagonist HS 378 on the development of arthritis. Adjuvant arthritis was induced in female Lewis rats with Mycobacterium butyricum suspended in paraffin oil. The delta-selective opioid antagonist HS 378 (0.5 to 8 mg/kg/day, i.p.) was administrated for 21 days. Drug treatment caused a significant reduction in the clinical severity of adjuvant arthritis, which was associated with less soft-tissue swelling and joint damage, as assessed by histology and radiology. Treatment with HS 378 produced a significant decrease in paw volume (~50%) and osteoclasts number (24-62%) in the ankle joint. This study provides evidence that the delta-opioid antagonist HS 378 attenuates the degree of chronic inflammation and tissue damage associated with adjuvant arthritis in the rat.

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THE EFFECT OF NOISE STRESS-INDUCED PAIN THRESHOLD ON CENTRAL MONOAMINERGIC NEURONES

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In this study, we attempted to investigate the antinociceptive mechanisms induced by noise stress in the formalin test. Norepinephrine (NE, 3~300 ug/kg, i.c.v.) and 5-HT (3~300 ug/kg, i.c.v.), and 5-HTP (50 mg/kg, i.p.) enhanced the antinociception of noise stress. However, NE showed statistical significance when high doses were used. Amphetamine potentiated the noise stress-induced analgesia at a low dose (0.5 mg/kg,s.c.), but not at higher dose (1~5 mg/kg, s.c.). The antinociception induced by noise stress was reversed by PCPA (200 mg/kg, i.p.), but not reversed by _-MT (100 mg/kg, i.p.). Prazosin (1 mg/kg) and clonidine (0.01~0.5 mg/kg, i.p.) could increase the pain threshold. However, yohimbine (1mg/kg, i.p.) was not effective. The above results suggested that the noise stress induced-analgesia might primarily be relation to the serotoninergic and postsynaptic noradrenergic neurones.

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SEX DIFFERENCES IN ANTAGONISM OF OPIOID ANTINOCICEPTION

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The goal of this study was to determine if sex differences in opioid receptor activation could explain sex differences in opioid antinociception. The opioid receptor-selective antagonists b-FNA (mu), nor-BNI (kappa), and NTI (delta) were administered i.c.v.; rats were injected systemically with the opioid agonists buprenorphine (mu), U69,593 (kappa), or SNC80 (delta), and then tested on a 52°C hotplate. Buprenorphine was more potent in males than females, whereas U69,593 and SNC80 were equipotent in males and females. B-FNA (5, 10 ug) shifted the buprenorphine dose-effect curve to the right similarly in females and males; nor-BNI and NTI did not shift the buprenorphine curve in either sex. Nor-BNI (1, 3 ug) shifted the U69,593 dose-effect curve farther to the right in females than males; b-FNA and NTI did not shift the U69,593 curve in either sex. NTI (10, 30 ug), b-FNA and nor-BNI did not shift the SNC80 dose-effect curve in either sex. These results confirm previous studies showing sex differences in mu opioid antinociception, but do not support the hypothesis that sex differences in mu opioid antinociception are due to differential opioid receptor activation between the sexes. However, greater nor-BNI antagonism of U69,593 in females suggests that females may have fewer kappa receptors or less efficient signal transduction than males. (Supported by NIDA Grant DA10284)

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PAIN CONTROL FOR KNEE ARTHORSCOPY: PERIPHERAL EFFECT OF KETAMINE.

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For demonstrating the peripheral analgesic effect of ketamine for arthroscopy in the synovia of knee joint, the aim of this study was designed to evaluate the analgesic effect of intra-articular ketamine injection after knee arthroscopy. In a double blind randomized study, 60 patients were assigned to three groups. Group A patients received saline 5ml intra-articular injection after closure of the surgical wound. Group B patients received ketamine 0.5 mg/kg intramuscular injection to rule out the systemic effect ketamine, while in group C, patients received ketamine 0.5 mg/kg in 5 ml of saline intra-articular injection after closure of the surgical wound. Pain score was evaluated by the visual analogue scale (VAS, 0 = no pain to 1 =worst pain) for 24 h at the position of extension t) and during active flexion of the knee joint (60^o). Rescue pethidine (1mg/kg) was given intramuscularly for pain relief on request every 4 hours postoperation. The time to first rescue analgesic request was recorded, and the total doses of pethidine were calculated. The results showed no differences on the VAS pain scores and total pethidine consumption at rest and during active range of motion (60^o) among three groups during the 24h observation. Ketamine was demonstrated to have peripheral analgesic effect with variable duration by measurements on pain and hyperalgesia in the past. However, in the present study, we failed to demonstrate the peripheral analgesic effect of ketamine for post-arthroscopy pain.

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DELTA-OPIOID RECEPTOR AND (1DMe)NPYF-MEDIATED EFFECTS IN SPINAL ANTINOCICEPTION

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Endogenous enkaphalinergic mechanisms have been suggested to mediate antinociceptive effects of neuropeptide FF (NPFF) in the spinal cord. A selective δ–opioid antagonist, naltrindole and δ-opioid agonist, DPDPE were used to study the role of enkaphalinergic systems in the antinociceptive actions of a synthetic NPFF analogue, (1DMe)NPYF, in models of acute, inflammatory and neuropathic pain in the rat. In the tail flick test, i.t. (1DMe)NPYF (5 nmol) produced antinociception that was significantly antagonised by naltrindole (28 nmol) pretreatment ($P<0.001$). (1DMe)NPYF (0.5 nmol) clearly potentiated the antinociceptive effect of i.t. morphine 7.8 nmol (2.5 μg). This effect was also decreased by the pretreatment of naltrindole (28 nmol) ($P<0.001$). In the carrageenan inflammation model, the antihyperalgesic effect of (1DMe)NPYF (5 nmol) in the paw flick test was abolished by pretreatment with naltrindole (28 nmol) ($P<0.01$). In the spinal nerve ligation neuropathic pain model, (1DMe)NPYF (5 nmol) significantly reduced mechanical allodynia. This effect was prevented by pretreatment with naltrindole (28 nmol) ($P<0.01$). This data suggests that activation of spinal δ-opioid receptors plays an important role in mediating the spinal antinociceptive effects of (1DMe)NPYF. This could be via release of enkephalins, as a recent study has shown that (1DMe)NPYF enhances met-enkaphalin release from the rat spinal cord. (1DMe)NPYF may also modulate the function of the δ-opioid receptors.

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DESENSITIZATION OF HUMAN DELTA OPIOID RECEPTOR INCREASES PHOSPHORYLATION OF $G_{\alpha i}/G_{\alpha o}$ PROTEINS

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Activation of delta-opioid and D2-dopaminergic receptors, that are endogenously expressed in the human neuroblastoma cells SK-N-BE, produce an inhibition of cAMP accumulation by 50 to 65 %. This inhibition is abolished by PTX pretreatment demonstrating the involvement of Gi and/or Go proteins. When SK-N-BE cells are pretreated for 15h in the presence of etorphine, we observed a robust reduction of the inhibitory actions of D2-dopaminergic receptors. Western-blot analysis revealed only minor reduction of $G_{\alpha i1}$ and $G_{\alpha i3}$ by 10 to 15% without any modification of $G_{\alpha i2}$ and $G_{\alpha o2}$ proteins level. ADP-ribosylation by PTX of these G_α subunits showed that etorphine pretreatment induced an increase in the ability of PTX to ADP-ribosylate $G_{\alpha i1}$ and $G_{\alpha i3}$ proteins. We next investigate whether etorphine pretreatment could induce a phosphorylation of G_α subunits. [^{32}P]Orthophosphoric acid labeling followed by immunoprecipitation of G_α subunits showed that etorphine increases phosphorylation of $G_{\alpha o}$ and $G_{\alpha i3}$ proteins.

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IMPAIRED OPIOID RECEPTOR INTERNALIZATION FOLLOWING CHRONIC MORPHINE TREATMENT

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Prolonged opioid exposure initiates multiple cellular adaptations (tolerance) that protect the cell from continued opioid stimulation. One of these mechanisms is the loss of functional cell surface receptors by their internalization, as observed for a variety of high efficacy opioids. However, recent studies have also shown that morphine lacks this regulatory property on both δ- and μ-opioid receptors. In the present study we demonstrate that, although chronic morphine treatment by itself has no effect on receptor regulation in both NG108-15 hybrid (endogenous δ-opioid receptors) and stably δ-opioid receptor transfected HEK293 cells, it completely prevents receptor internalization following subsequent activation by a high efficacy opioid, such as etorphine or DADLE. The time- and dose-dependency of this chronic morphine reflects that observed for the development of tolerance/dependence to adenylyl cyclase regulation and is blocked by naloxone and pertussis toxin pretreatment. In addition, chronic morphine treatment also heterologously affected internalization of other G protein-coupled receptor species, *e.g.* muscarinic M4 acetylcholine receptors, suggesting a more general mechanism. In this respect, we found that chronic morphine treatment prevents redistribution of β-arrestin from the cytosolic fraction to the plasma membrane, as seen after acute receptor activation in naive cells. These results identify the regulatory pathway of clathrin-mediated endocytosis of G protein-coupled receptors as a novel target of chronic morphine action and further suggest that state of opiate tolerance/dependence is characterized by an overall impaired agonist-mediated regulation of inhibitory receptors.

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TYROSINE PHOSPHORYLATION OF THE KAPPA OPIOID RECEPTOR ENHANCES AGONIST EFFICACY

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The goal of this study was to determine the role of highly conserved tyrosine residues in the putative cytoplasmic domains of opioid and other G protein-coupled receptors. The kappa opioid receptor (KOR) was expressed in *Xenopus* oocytes and the intrinsic insulin receptor tyrosine kinase activated. Kappa opioid receptor activation by the agonist U69,593 produced a strong increase in potassium current through co-expressed G protein-gated inwardly rectifying potassium channels ($K_{IR}3$). Brief treatment with insulin caused a 60% potentiation of the KOR-activated response. The insulin-induced increase in kappa opioid response was blocked by the tyrosine kinase inhibitor genistein. In contrast, insulin had no effect on the basal activity of $K_{IR}3$ suggesting that KOR was the target of the tyrosine kinase cascade. Mutation of tyrosine residues to phenylalanines in either the first or second intracellular loop of KOR to produce KOR(Y87F) and KOR(Y157F) had no effect on either the potency or maximal effect of U69,593. However, neither KOR(Y87F) nor KOR(Y157F) mediated responses were potentiated by insulin treatment. Insulin pretreatment shifted the dose response curve of U69,593 activation of KOR by increasing the maximal response without changing the EC50 of U69,593. These results suggest that insulin increased the efficacy of kappa opioid receptor activation by phosphorylating two tyrosine residues in the first and second intracellular loops of the receptor. Thus, the potentiation of G protein coupled receptor signaling by tyrosine phosphorylation may provide an important mechanism of receptor modulation. Supported by USPHS grant DA11672.

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ON THE MECHANISM OF ERK REGULATION BY MU OPIOID RECEPTORS IN C6 GLIOMA CELLS. M. M. Belcheva, L. M. Bohn, P. Haas and C. J. Coscia, Dept. Biochem. & Mol. Biol., St. Louis Univ. Sch. Of Med., St. Louis, MO 63104.

We have recently reported on the mechanism involved in kappa opioid activation and chronic mu inhibition of the MAP kinase phosphorylation cascade in rat C6 glioma cells. Here we describe experiments that delineate endogenous and overexpressed mu opioid receptor signaling to extracellular signal-regulated kinase (ERK) in C6 cells. In addition to the mu chronic inhibitory effects, mu selective opioid agonists stimulated endogenous receptor-mediated ERK phosphorylation maximally within 1-5 min exposure. Endomorphin or morphine induced a two-fold increase in ERK phosphorylation. By overexpressing dominant-negative or sequestering mutants, we obtained evidence that mu opioid ERK activation is Ras-dependent and is transduced by G protein beta gamma subunits. Wortmannin had no effect while dynamin dominant suppressor mutant (K44A) attenuated mu opioid receptor stimulation of ERK activity in mu opioid receptor transfected cells. Kappa stimulation of ERK phosphorylation was insensitive to K44A. The data suggest that although mu and kappa opioid signaling to ERK differ, they converge upstream of Ras in C6 cells. Supported by NIDA grant DA05412.

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MU OPIOID RECEPTOR SPLICED VARIANT, MOR1D, INTERNALIZES AFTER MORPHINE TREATMENT IN HEK-293 CELLS.

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The mouse mu opioid receptor (MOR1) has been shown to traffic from the plasma membrane to small vesicles in HEK-293 cells upon treatment with opioid agonists such as DAMGO. However, these receptors do not internalize after treatment with the opiate alkaloid, morphine. Recently, the MOR1 was shown to be subject to mRNA modifications leading to a number of spliced variants of the receptor (*Pan et al., Mol. Pharm., 56, 1999*). These modified receptors (MOR1C, MOR1D and MOR1E) differ in their C-terminal domains, however, the functional relevance of these variations has not been elucidated. We have generated GFP-tagged forms of these receptors (MOR1C, MOR1D, and MOR1E) to allow for visualization of receptor-membrane dynamics after agonist treatment. Our observations indicate that the MOR1D-GFP internalizes in HEK-293 cells after DAMGO treatment (1μM, 30 min) similar to the MOR1-GFP. Interestingly, the MOR1D-GFP also internalizes into small vesicles after morphine treatment (1μM, 30 min) whereas the MOR1-GFP does not. These observations suggest that modifications of the MOR1 that arise from mRNA splicing may attribute to differential regulation of signalling by agonists. *Supported by NIH grants DA006023 (L.M.B.); HL61365 (L.S.B.); DA00296 (Y.X.P.); DA02615, DA00220 (G.W.P.); and NS19576 (M.C.G.).*

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EXAMINATION OF SEVERAL ALTERNATIVELY SPLICED MU OPIOID RECEPTOR (MOR-1) ISOFORMS IN FUNCTIONAL ASSAYS.

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Recently, several alternatively spliced isoforms of the mu opioid receptor have been cloned. These new variants share the same protein structure as MOR-1 through the seven transmembrane spanning domain region but differ in the intracellular C-terminal region. In stably transfected Chinese hamster ovary (CHO) cells ^{3}H-DAMGO binds with high affinity to all variants. Morphine and M6G have modest affinity for the clones, as do a variety of other mu ligands, competing binding with K_i values under 10 nM. Subtle differences in affinity were observed between some clones with the endogenous peptides dynorphin-A and β-endorphin. Using a variety of mu ligands, including several endogenous peptides, we assessed function of the receptors by measuring the agonist stimulated accumulation of a radiolabeled nonhydrolyzable analog of GTP, GTPγS, in stably transfected CHO cells. Differences in agonist stimulation of the Erk-1 and Erk-2 signalling pathway were also investigated in the various clones.

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INVOLVEMENT OF MAPK PATHWAYS IN THE REGULATION OF HIPPOCAMPAL OPIOID PEPTIDES EXPRESSIONS INDUCED BY KAINIC ACID

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KA increased the proenkephalin as well as prodynorphin mRNA expression in the rat hippocampus, which was decreased by pre-administration of cycloheximide (CHX; a protein synthesis inhibitor). KA also increased c-fos as well as c-jun mRNA levels. However, CHX further enhanced KA-induced c-fos and c-jun mRNA levels. Additionally, KA increased the phosphorylation of ERK, JNK and p38 MAPK, which was attenuated by CHX. Furthermore, CHX decreased KA-induced c-Fos and c-Jun protein expressions. Interestingly, CHX itself increased phosphorylation of CREB, which was abolished by KA administration. Our results suggest that the phosphorylation of ERK, JNK or p38 MAPK, but not CREB phosphorylation, appears to be involved in the up-regulation of proenkephalin and prodynorphin gene expression in the rat hippocampus.

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CONSTITUTIVE ACTIVITY AND ADENYLYL CYCLASE INHIBITION BY MU AND DELTA OPIOID RECEPTORS IN HEK293T CELLS

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Several 7-transmembrane domain G protein-linked receptors show constitutive activity. In this study we demonstrate constitutive activity of mu and delta opioid receptors expressed in HEK293T cells and examine some properties of these receptors. Rat mu and/or delta opioid receptor cDNA and LH receptor cDNA were transiently transfected into HEK293T cells. Accumulation of cAMP was measured following 30 min incubation of cells with the LH agonist hCG in the absence of opioid ligand. Cells expressing mu or delta receptors together with the LH receptor showed a reduced cAMP accumulation in response to hCG than cells expressing the LH receptor alone. This constitutive activity correlated with receptor number (50-6000 fmol/mg) up to a maximal inhibition of 40% of the hCG stimulated cAMP level. Constitutive activity at the delta receptor was inhibited by ICI174864. Agonist-mediated inhibition of the hCG stimulated cAMP accumulation also correlated with receptor number for both mu and delta receptors. Mu and delta agonist action was not additive, nor was constitutive activity of co-expressed mu and delta receptors, indicating that the different opioid receptors may be acting through the same G proteins.

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ANATOMICAL INTERACTION BETWEEN MOR AND MULTIPLE G PROTEIN ALPHA SUBUNITS.

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The interaction between the mu opioid receptor (MOR) and G protein signaling complexes dictate the consequences of MOR activation. However the subcellular relationship between MOR and G protein alpha subunits remain poorly understood, particularly *in vivo.* Here we investigated the ultrastructural distribution of the G protein alpha subunits, Gi, Go and Gz with respect to MOR in the periaqueductal gray (PAG) using dual-labeling immunohistochemistry. In addition, the distribution of these G proteins with respect to MOR was followed after etorphine-produced internalization. Immunogold-silver labeling for each G protein revealed a predominant distribution in neuronal dendrites that was highly overlapping with MOR immunolabeling. Coexistence of G protein and MOR immunolabeling was more common with Gi then Gz, and least common with Go. After the administration of etorphine, immunoreactivity for each of the three G proteins could be seen clustered next to MOR-immunoreactive endosomes. These observations reveal that MOR has the capacity to interact with different G proteins within the same population of neurons. It may be inferred that through these associations, MOR could function to modulate heterogeneous signaling pathways.

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KETAMINE POTENTIATES MORPHINE ACTIVATION OF THE MAPK PATHWAY.

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Ketamine, a NMDA receptor antagonist, enhances opioid induced analgesia and prevents hyperalgesia1. It interacts with recombinant opioid receptors to decrease the affinity of opioid ligands2. Recently we showed that activation of opioid receptors leads to the phosphorylation of mitogen activated protein kinase (MAPK)3 in SKNSH cells expressing endogenous receptors. In this study we examined the effect of ketamine on morphine induced increase in the phosphorylation of MAP kinase in these cells. SKNSH cells were incubated with morphine, ketamine or a combination of both and analyzed by Western blotting using antisera that recognizes phosphorylated MAPK. We find a dose-dependent increase in MAPK phosphorylation by morphine ($p<0.001$). Treatment with ketamine alone does not substantially increase the level of phosphorylation. Interestingly, treatment with morphine in the presence of ketamine leads to a robust increase (~4 fold) in MAPK phosphorylation ($p<0.05$). Our results show that ketamine modulates the effects of morphine on the activation of the MAPK pathway.

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μ-OPIOID RECEPTOR ACTIVATION OF KIR 3 IS SUPPRESSED BY THE NEUROTROPHIN BDNF

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Tyrosine phosphorylation results in rapid modulation of neuronal excitability in response to extracellular stimuli. The G-protein coupled inwardly rectifying potassium channel (Kir) integrates receptor-mediated signal transduction pathways, altering potassium influx to modify neuronal signalling. In *Xenopus* oocytes, Kir activity can be differentially modulated by insulin, brain-derived neurotrophic factor (BDNF), and the G-protein coupled μ opioid receptor (μOR). To characterize the regulation of the μOR by tyrosine kinases stimulated by insulin or BDNF, μOR, trkB, and either hetero- or homomultimers for the Kir 3.1 and 3.2 were expressed in *Xenopus* oocytes. Two electrode voltage clamp was used measure potassium influx in the presence of μOR agonist DAMGO. Pretreating oocytes in BDNF was found to suppress the μOR-induced enhancement of potassium influx by greater than 50% ($p<0.05$). In contrast, insulin potentiated the potassium influx by about 160% ($p<0.05$). To investigate the possibility that μOR tyrosine phosphorylation generates these disparate effects, site-directed mutagenesis was used to create μORs with key tyrosine residues mutated to phenylalanines (Y96F, Y106F, Y166F, and Y336F). BDNF was applied to *Xenopus* oocytes expressing the mutant μORs, trk B, and Kir channels. The results of this study imply that different tyrosine kinase-coupled signals differentially modulate the μOR-induced activation of Kir3. (supported by USPHS grant DA 11672.)

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THE ERKSOME RELATIONSHIP BETWEEN SRC, MAPK AND OPIOID RECEPTORS IN SIGNALING AND REGULATION.

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Among the myriad of mechanisms proposed to modulate the sensitivity and function of G protein-coupled receptors (GPCRs), only one – receptor phosphorylation -- remains a consistent theme across numerous receptor types. While a role for G protein receptor kinases (GRKs) in opioid receptor (OR) regulation has been exhaustively investigated, other protein kinases, including extracellular signal-regulated protein kinases (ERKs) and protein and receptor tyrosine kinases (PTKs and RTKs) appear to be importantly involved in regulating agonist-stimulated opioid receptors. Our laboratory has determined that opioid-activated, Src-like protein tyrosine kinases are responsible for mediating the tyrosine phosphorylation of the delta opioid receptor in a time- and concentration-dependent manner. In addition, tyrosine phosphorylation of the DOR is necessary for agonist-dependent desensitization, internalization and down-regulation to occur in CHO-δOR cell lines. Furthermore, it appears that two tyrosines in the C-terminus are the predominant recipients of Src-dependent phosphorylation, since the mutation of either site to Phe greatly increases the resistance of the δ–OR to desensitization and internalization. Surprisingly, the loss of C-terminal tyrosines also has a profound effect on acute delta opioid receptor signalling, as these tyrosine-deficient mutants can no longer signal via the MAPK pathway. Our data will demonstrate that opioid receptors are capable of acting as scaffolds for the formation of a multi-protein complex that is required for both Ras translocation and ERK activation. Lastly, we will describe that significant changes in ERK and Src activity occur during opioid "withdrawal", which suggests a global importance of these two Gβγ-mediated signaling events in opioid receptor signalling, desensitization and recovery (K01-DA00437 to HKK).

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A PHOSPHATIDYL-ETHANOLAMINE BINDING PROTEIN 23kDa-PEBP ENHANCES Gi/o PROTEIN MEDIATED SIGNALLING OF OPIOID RECEPTORS

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The μ opioid receptor (MOR) has been proposed to be associated with a 23kDa-phosphatidyl-ethanolamine binding protein (23kDa-PEBP). To characterize the role of PEBP in MOR function we used heterologous expression of the human μ opioid receptor MOR1 and the 23-kDa-PEBP in Xenopus laevis oocytes as well as in NIH3T3 and HEK293 cells. The 23kDa-PEBP has a stimulatory effect on μ opioid receptor mediated agonist induced G protein coupling to G protein activated inwardly rectifying potassium channels (KIRs) in oocytes and enhances agonist mediated inhibition of adenylyl cyclase in HEK293 cells. No direct interaction of MOR and 23kDa-PEBP could be detected using *Yeast Two-Hybrid* system. Stimulation of agonist induced channel activity was also observed when other Gi/o protein coupled receptors were coexpressed with the 23kDa-PEBP (e.g. human opioid receptor (hDOR) and somatostatin receptor (hSSTR2). In addition NIH3T3 stably transfected with 23kDa-PEBP but not mock transfected cells displayed a marked activation of mitogen activated protein kinase (MAPK p42/44) under serum-starved conditions (0,5% FCS) which could be blocked by pertussis toxin.These data indicate a novel functional interaction of 23kDa-PEBP with Gi/o protein coupled receptor systems on the level of G protein.

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IDENTIFICATION OF GRK2 SITES FOR AGONIST-STIMULATED δ OPIOID RECEPTOR PHOSPHORYLATION AND DESENSITIZATION

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Truncation (Δ15) or substitutions (T358A, T361A, or S363A single mutant or triple mutant M3) at mouse DOR cytoplasmic tail caused 80–100% loss of opioid-stimulated receptor phosphorylation, indicating that T358, T361 and S363 all contribute and are cooperatively involved in agonist-stimulated DOR phosphorylation. Coexpression of GRK2 strongly enhanced agonist-stimulated phosphorylation of the wild-type DOR (WT), but Δ15 or M3 failed to show any detectable phosphorylation under these conditions. Furthermore, coexpression of GRK2 resulted in a remarkable reduction of opioid-induced, DOR mediated inhibition of cellular cAMP accumulation, while removal the last 15 amino acids or mutation of T358, T361, or S363 strongly attenuated the effect of GRK on the responsiveness of DOR. Agonist induced receptor phosphorylation was severely impaired by substitution of either T358 or S363 with aspartic acid residue but phosphorylation of T361D mutant was comparable to that of WT. In the presence of exogenously expressed GRK2, phosphorylation levels of T358D and S363D mutants were approximately half of that of WT, whereas significant phosphorylation of T358/S363 double-point mutant was not detected. These results indicate that both T358 and S363 residues at the DOR carboxyl terminus are capable of serving cooperatively as phosphate acceptor sites of GRK2 in vivo. Taken together, we have demonstrated that agonist-induced opioid receptor phosphorylation occurs exclusively at two phosphate acceptor sites (T358 and S363) of GRK2 at the DOR C-terminus.

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INDUCTION OF MULTIPLE EFFECTS ON ADENYLYL CYCLASE AND CREB PHOSPHORYLATION UPON EXPOSURE TO MORPHINE OF CELLS TRANSFORMED WITH THE μ-OPIOID RECEPTOR

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Adenylyl cyclase regulation represents an important part of the opioid response. It has been implicated in the control of expression levels of various transcription factors. In this regard, we have found that chronic morphine treatment of HEK 293 and Neuro2A cells stably transformed with the μ-opioid receptor results in upregulation of adenylyl cyclase activity and stimulation of cAMP response element binding protein (CREB) phosphorylation, thought to be mediated by protein kinase A (PKA) activation. By contrast, acute exposure to morphine decreases AC activity with a rapid and transient increase in CREB phosphorylation. Cotransfection of Gβγ scavengers prevents AC superactivation and CREB phosphorylation. These data provide information about various molecular mechanisms through which administration to opiates alter gene expression in specific target neurons and thereby induces tolerance and dependence. *Supported by a State Scholarship Foundation of Greece to G.M and YPER6 grant to Z.G.*

Sun54

INSULIN POTENTIATES OPIOID ACTIVITY THROUGH TYROSINE PHOSPHORYLATION OF THE MU OPIOID RECEPTOR.

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Insulin activates a tyrosine kinase cascade, thereby modulating the activity of a wide range of neurosystems. *Xenopus* oocytes were injected with mRNA to express the rat mu-opioid receptor (μOR) and the heteromultimeric potassium channel, KIR 3.1/3.2. Pretreatment with a maximally effective concentration of insulin (8 μM) insulin caused a 40% suppression in the basal potassium current and a 155% potentiation of response to the μ-opioid agonist, DAMGO. Insulin-induced potentiation of the DAMGO response was concentration-dependent and reversible after 1 hr, while alteration of the potassium response persisted over 2 hr. Insulin pretreatment did not change the affinity of the μOR for DAMGO, as the EC_{50} (and 95% C.I.) values for DAMGO-induced K^+-currents were 41.8 (19.3-90.8) nM in control and 44.5 (23.5-84.1) nM in treated oocytes. Moreover, specific labeling of cell surface expression of μOR with [^{3}H]CTOP in whole-oocyte binding experiments revealed no significant differences between control and insulin-pretreated oocytes, suggesting the potentiation in the DAMGO response was not due to a change in receptor number. Phosphorylation of tyrosine residues on the putative intracellular loops of the μOR was demonstrated by testing four point-mutated receptors, using site-directed mutagenesis to replace tyrosine with phenylalanine to create μY96F, μY106F, μY166F and μY336F. None of these mutations significantly altered the EC_{50} value for DAMGO, and insulin pretreatment still potentiated the effect of 1 μM DAMGO in oocytes containing μY96F and μY336F by 137 ± 10% and 124 ± 8%, respectively. However, insulin incubations with oocytes containing μY106F and μY166F did not significantly potentitate the DAMGO response, suggesting these two amino acids were responsible for the insulin-induced opioid potentiation. These findings support a rapid modulatory role by insulin on opioid signal transduction, possibly through the phosphorylation of the μOR at tyrosines 106 and 166 by an insulin-induced kinase. (Supported by USPHS grants DA07278 and DA11672.)

Sun55

STRATEGIES FOR STUDYING THE ROLES OF OPIOID RECEPTOR SIGNALING

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Previous published results from our laboratory concerning the interactions of opioid receptors with G proteins, have shown that the third intracellular loop (i3) is responsible for coupling to G proteins and inhibition of adenylyl cyclase. Based on these observations and in order to design and develop analogues that could potentially block or activate selectively in vivo, the interactions of these receptors with G proteins, or other downstream signaling components, we constructed a minigene encoding the third intracellular loop of the δ-opioid receptor ($pcDNA_3$-di.3). Transient transfection of COS-7 cells with the cDNA of the δ-opioid receptor in the presence or not of the $pcDNA_3$-di.3, have shown no changes in receptor expression as assessed by specific [^{3}H]diprenorphine binding, but alterations in cAMP accumulation in intact cells. Similar results were also observed when cotransfection of HEK293 cells, stably transformed with the μ-opioid receptor, with the $pcDNA_3$-di.3 were performed. Our results indicate that the i3 minigene, is able to interact specifically with the G_i population of G proteins, for both μ-and δ-opioid receptors in intact cells. These observations underlie the significance of the i3 loop for signal transfer of both μ- and δ-opioid receptors, and suggest a strategy whereas the receptor-G protein interface may represent a target for novel receptor drug development. (Supported by YPER6 and Demoereuna to Z.G)

Sun56

OPIOID PRETREATMENT INCREASES CYCLIC GMP LEVELS IN SH-5YSY CELLS.

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The development of tolerance to opioids has been studied extensively in recent years. However, the cellular mechanisms underlying tolerance have yet to be fully elucidated. The nitric oxide/cyclic GMP system has been studied as a potential mediator in the development of tolerance. Cyclic GMP (cGMP) is produced *in vivo* by guanylyl cyclase (GC), which is activated by nitric oxide. Previous research in our lab demonstrated in mice that LY-83,583, a soluble GC inhibitor, blocked the development of acute antinociceptive tolerance to i.c.v. administered morphine. The aim of this study was to characterize changes in cGMP levels after opioid pretreatment in a cellular model. SH-SY5Y cells, a human neuroblastoma cell line that mainly express μ opioid receptors, were used. The cells were cultured in the presence of the μ–selective agonist, DAMGO, for 15 min and 24 hr. After 1 μM DAMGO pretreatment the cyclic nucleotides were extracted with trichloroacetic acid and cGMP levels were quantitated by an enzyme immunoassay. The results demonstrate that cGMP levels were greater in cell extracts from cells pretreated with 1 μM DAMGO for 24 hr as compared to cell extracts from control or 15-min pretreated cells. This increase was not due to an increase in receptors because 1 μM DAMGO treatment for 24 hr decreased the number of μ receptors by approximately 80% without altering the K_D value. These findings provide preliminary evidence that treatment of SH-5YSY cells with opioids produces changes in the NO/cGMP system. Future studies will examine the role that these changes may have in the development of desensitization and tolerance. (Supported by grants DA03742 and DA07232.)

Sun57

DIFFERENTIAL INTERACTIONS BETWEEN OPIOID AND α2 ADRENERGIC AGONISTS FOR ENHANCING [^{32}P] GTP AZIDOANILIDE (GTP-AA) INCORPORATION INTO SPINAL Go1α.

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Opioid and α2 adrenergic agonist activation of spinal G proteins may contribute to analgesic synergism between agonists. Agonist-enhanced incorporation of [^{32}P]GTP-AA into G protein α subunits was used to identify and quantify spinal G proteins activated by opioid and α2-adrenergic receptors. Mu (fentanyl, DAMGO, and PL017l), delta (DPDPE and SNC80) or kappa (U50-488H) opioids, or the α2 agonists (clonidine, UK14,304 and dexmedetomidine) were incubated with mouse spinal neuronal membranes and [^{32}P]GTP-AA. After UV irridation, proteins were separated by SDS PAGE. All the agonists increased [^{32}P] labeling of at least two G protein α subunits. One was identified as $G_{o1\alpha}$. Enhanced photolabeling by DAMGO or dexmedetomidine was blocked by naloxone or idazoxan, respectively. Incorporation of [^{32}P] into $G_{o1\alpha}$ was additive after coincubation of opioids (DAMGO, DPDPE or U50-388) with clonidine and of DAMGO (but not DPDPE or U50-188) with dexmedetomidine. These results suggest that atimulation of spinal opioid and α2 adrenergic receptors activates $G_{o1\alpha}$ and this activation is additive with some agonist combinations. (Supported by DA07972)

Sun58

TRKB ACTIVATION BY BDNF INHIBITS THE G PROTEIN GATED INWARD RECTIFIER KIR3 BY TYROSINE PHOSPHORYLATION OF THE CHANNEL.

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G protein activated inwardly rectifying potassium channels (Kir3) are widely expressed throughout the brain, and regulation of their activity modifies neuronal excitability and synaptic transmission. We show that the neurotrophin BDNF, through activation of TrkB receptors, strongly inhibited the basal activity of Kir3. This inhibition was subunit dependent as functional homomeric channels of either Kir3.1 or Kir3.4 were significantly inhibited, whereas homomeric channels composed of Kir3.2 were insensitive. The general tyrosine kinase inhibitors genistein, Gö6976, and K252a, but not the ser/thr kinase inhibitor staurosporine blocked the BDNF-induced inhibition of the channel. BDNF was also found to directly stimulate channel phosphorylation because Kir3.1 immunoprecipitated from BDNF-stimulated cells showed enhanced labeling by antiphosphotyrosine-specific antibodies. The BDNF effect required specific tyrosine residues in the amino-terminus of Kir3.1 and Kir3.4 channels. Mutations of either Y12, Y67, or both, in Kir3.1 or mutation of either Y32, Y53, or both, of Kir3.4 channels to phe significantly blocked the BDNF-induced inhibition. The insensitive Kir3.2 was made sensitive to BDNF by adding a tyrosine (D41Y) and a lysine (P32K) upstream to generate a phosphorylation site motif analogous to that present in Kir3.4. These results suggest that neurotrophin activation of TrkB receptors may physiologically control neuronal excitability by direct tyrosine phosphorylation of the Kir3.1 and Kir3.4 subunits of Kir 3. Supported by DA11672.

Sun59

THE OPIOID-ACTIVATED POTASSIUM CHANNEL (KIR 3) IS INHIBITED BY PLA2 GENERATED EICOSANOIDS.

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We previously showed that activation of the mu opioid receptor strongly increased Kir 3 channel current, whereas, activation of the endothelin A receptor inhibited the Kir 3 response evoked by mu opioid receptor activation. The endothelin-1 (Et-1) mediated inhibition was mimicked by arachidonic acid and blocked by the phospholipase A2 inhibitor AACOCF3. Consistent with a possible phospholipase A2-mediated mechanism, the endothelin-1 effect was blocked by calcium chelation with BAPTA-AM. The magnitude of the inhibition of Kir3 was channel subtype-specific: heteromultimers composed of Kir 3.1 and Kir 3.2 or Kir 3.1 and Kir 3.4 were significantly more sensitive to the effects of endothelin-1 than heteromultimers composed of Kir 3.1 and Kir 3.5.

In this study, we generated functional homomeric Kir 3 channels by mutating a specific residue in the pore of Kir 3.1, Kir 3.2 and Kir 3.4. We show that the endothelin-induced inhibition of the mu opioid receptor response is channel subunit-specific. The channel response to mu opioid receptor activation by DAMGO was significantly inhibited by Et-1 activation of the endothelin A receptor in functional homomeric channels of either Kir 3.2 or Kir 3.4 . In contrast, Et-1 activation of the endothelin A receptor potentiated the mu opioid response to DAMGO when coexpressed with the Kir3.1 functional homomer. The data suggest that endothelin receptor activation may modulate the mu opioid response by a direct effect on Kir 3 in a channel subtype-specific fashion. Supported by DA11672.

Sun60

DIFFERENTIAL STIMULATION OF [^{35}S]GTPγS BINDING BY DELTA AGONISTS AT A TRP284 MUTANT OF THE HUMAN DELTA OPIOID RECEPTOR

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We examined the stimulation of [^{35}S]GTPγS binding at the wild type and a mutant of the human delta opioid receptor in which Trp284 was replaced with Leu (W284L), each expressed in Chinese hamster ovary cells. Receptor density between the two stable cell lines was balanced by the addition of untransfected CHO cell membranes. SNC80 and DPDPE, two delta-selective agonists, were examined. Although SNC80 stimulated GTPγS binding in W284L mutant cell membranes 200% higher than in wild type receptor cell membranes (E_{max}), its potency and affinity were significantly reduced in W284L cell membranes. The potency and E_{max} of DPDPE remained unchanged by the point mutation. These results indicate that Trp284 in the human delta opioid receptor plays a role in SNC80-mediated G-protein stimulation, whereas it has no effect on DPDPE-mediated GTPγS binding. (This research supported by the ADCRC and NIDA grants.)

Sun61

MU-, KAPPA$_3$- AND ORL-1-RECEPTOR MEDIATED ACTIVATION OF p42 AND p44 MITOGEN-ACTIVATED PROTEIN KINASES IN HUMAN NEUROBLASTOMA CELLS.

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Mu (DAMGO), kappa$_3$ (naloxone benzoylhydrazone; NalBzoH) and ORL-1 (orphaninFQ/nociceptin; OFQ/N) receptors induce rapid, short-term and dose-dependent activation of p42/p44 mitogen-activated protein kinases (MAPK) in BE(2)-C and SH-SY5Y human neuroblastoma cell lines that endogenously express mu, delta, kappa$_3$ and ORL-1 receptors. DAMGO- and NalBzoH-mediated increases in MAPK activity were blocked by CTAP and Mr2266, respectively. The dose-dependent activation of MAPK by DAMGO, but not by NalBzoH, in CHO-MOR cells confirmed the kappa$_3$ receptor mediated action of NalBzoH. All agonist-mediated increases in MAPK activity were completely abolished upon pretreatment with pertussis toxin and were also blocked by the phosphotidylinositol 3-kinase inhibitor, wortmannin. DAMGO- and OFQ/N-mediated MAPK activation was also attenuated by the protein kinase C inhibitor, chelerythrine, while NalBzoH-mediated MAPK activition was blocked by the protein kinase A inhibitor, H-9. Chronic activation (1-3 days) of mu and kappa$_3$, but not ORL-1 receptors, led to an upregulation of tyrosine hydroxylase (an enzyme known to be upregulated as an intracellular adaptation upon chronic morphine exposure). These responses were attenuated upon pretreatment with the MEK-1 inhibitor, PD98059, suggesting a role for MAPK in the development of tolerance and dependence upon chronic activation of these receptors. This work was supported by a grant from the National Institutes of Health (DA10738).

Sun62

UNIQUE INTERACTIONS OF SNC80 WITH THE HUMAN DELTA OPIOID RECEPTOR (hDOR)

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The delta selective diarylmethylpiperazine opioids (SNC80 analogues) have an unusual structure-activity relationship relative to other opioid drugs. Several lines of evidence show that SNC80 analogues might activate the hDOR through a unique conformational mechanism. SNC80 and DPDPE are equipotent full agonists in the [^{35}S]GTPγS binding assay in hDOR/CHO cell membranes, but their analgesic potencies are different in a mouse tail-flick assay. Mutation of Trp^{284} in the hDOR to Leu selectively decreases the binding affinity of SNC compounds while selectively increasing their intrinsic activity in [^{35}S]GTPγS binding assays. Truncation of 34 C-terminal amino acids of the hDOR blocks DPDPE mediated downregulation while SNC80 still causes a significant (42%) reduction of [^{3}H]NTI binding to CHO cell membranes stably expressing the truncated hDOR. The truncated hDOR is phosphorylated by chronic SNC80 treatment but not by chronic DPDPE treatment. These findings suggest that SNC80 induces or recognizes a unique hDOR conformation that interacts differently with downstream effectors (G proteins, kinases and/or arrestins) than the conformation recognized by other delta selective agonists. (Supported by grants from ADCRC and NIDA.)

Sun63

CHARACTERIZING THE KINASE PKU-α: A POTENTIAL NOVEL MODULATOR OF THE MU OPIOID RECEPTOR.

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Endogenous opioid peptides, such as endorphins, and exogenous opioids, such as morphine, exert various biological effects, including analgesia, respiratory depression, neurotransmitter and hormone release, and euphoria. Opioids act upon three receptor proteins, mu, kappa, and delta, to mediate these effects. Much is known about acute opioid affects. However, receptor-mediated long-term effects of opioid drugs are still not fully understood, especially the intracellular factors that modulate receptor-mediated signal transduction processes. Because the mu receptor is the major cellular target of the most commonly used opioid drugs, we screened a cDNA expression library to identify proteins that might interact directly with the mu receptor. Using a GST-fusion protein containing the carboxyl terminus of the human mu opioid receptor, we identified a novel protein kinase termed PKU-α. This kinase is a member of a growing family of eukaryotic kinases related to the plant TOUSLED kinase involved in normal flower and leaf development. PKU-α contains a consensus serine/ threonine catalytic domain and a putative nuclear localization signal (NLS) but whose function remains unknown. We are currently studying the physiological relevance of the interaction between the mu receptor and this kinase. Preliminary results of kinase assays using myelin basic protein as the substrate indicate that the enzymatic activity of PKU-α is positively regulated by receptor activity.

Mon01

HOMODIMERIZATION OF THE μ-OPIOID RECEPTOR MOR1 IN HEK 293 CELLS.

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It has been shown that GPCR´s can interact at the molecular level to form homo- or heterodimers. In the present study, we have examined dimerization of rat MOR1 in stably transfected HEK 293 cells. Immunoblot analysis of membrane extracts of MOR1-expressing cells revealed a predominant receptor band at 70-80 kDa. Interestingly, we also observed an additional band with a higher molecular mass migrating at 140 kDa. Enzymatic deglycosylation reduced the size of the 70-80 kDa protein to 45 kDa and the size of the 140 kDa protein to 90 kDa suggesting that the high molecular mass protein is a homodimeric form of the receptor. The homodimers were stable under reducing conditions, e.g. in the presence of dithiothreitol or ß-mercaptoethanol. The degree of dimerization was agonist independent. We also examined the ability of the mouse MOR1 to form dimers in HEK 293 cells. Like the rat MOR1, the mouse MOR1 also exists as a homodimer. However unlike MOR1, the recently identified mouse MOR1 splice variants, MOR1D and MOR1E did not assemble as homodimers at the plasma membrane. These findings suggest that the COOH-terminal tail of MOR1 may be involved in receptor dimerization.

Mon02

IDENTIFICATION OF POLYMER, DIMER AND MONOMER FORMS OF HUMAN KAPPA OPIOID RECEPTOR: INVOLVEMENT IN RECEPTOR BINDING AND ACTIVATION.

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Kappa opioid receptors have been reported to exist as homo- and hetero-dimers. However, the involvement of these conformations in receptor binding and activation remains unclear. In the present study, changes in the conformation of human kappa opioid receptor (hKOR), stably expressed in CHO cells, were investigated and correlated with receptor function. Immunoprecipation followed by Western blotting, showed that the hKOR existed in three different forms: monomer, dimer and polymer. Dithiothreitol treatment of cells or membranes showed that dimers and polymers were formed from monomers by SH group interactions. More interestingly, Scatchard analysis demonstrated that the three forms bound [^{3}H]U69,593 with similar K_D and B_{max} values, suggesting that the SH groups involved in receptor dimerization and/or polymerization were not located within the agonist-binding site. Using iodoacetamide, we showed that SH groups, different from those involved in receptor dimerization and/or polymerization, were located within the agonist-binding site. Moreover, neither the dimer nor the monomer forms inhibited cAMP accumulation, whereas agonist activation of the polymer form allowed an inhibition of adenylyl cyclase activity. These results suggest that the polymer form of hKOR is a ligand-binding form, which triggers the agonist-mediated signal transduction. These findings are the first to indicate the importance of a polymeric form of the human kappa opioid receptor. They point to a new view, which could represent a novel scheme for ligand binding, receptor activation and receptor-function modulation. (Supported by grant DA03742.)

Mon03

CONSTITUTIVELY ACTIVE MUTANTS OF THE μ OPIOID RECEPTOR

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The highly conserved DRY motif is thought to play an important role in GPCR activation. In this study, we determined whether mutation of the Asp residue of the DRY motif in the rat μ opioid receptor led to constitutive activity of the receptor. Asp164(3.49) was mutated to 11 different amino acids (H, Q, Y, M, S, N, I, V, L, A and E) and, when transiently expressed in HEK293 cells, each mutant receptor displayed little or no detectable [^{3}H]diprenorphine binding, except for the D164E mutant. Addition of naloxone to the culture medium greatly enhanced [^{3}H]diprenorphine binding to five mutants (H, Q, Y, M and S). These five mutants, when stably expressed in CHO cells, exhibited greatly enhanced basal [^{35}S]GTPγS binding, up to 2.4-fold of that of the wildtype, comparable to the activated level of the wildtype. The agonist-independent activities were related to receptor expression levels. Treatment with pertussis toxin completely abolished agonist-independent elevation in [^{35}S]GTPγS binding, indicating constitutive activation of Gαi/Gαo by the mutants. The five mutants had higher affinities for the agonist DAMGO than the wildtype, but displayed similar affinities for the antagonist [^{3}H]diprenorphine as the wildtype. Thus, mutation of Asp164(3.49) to His, Glu, Tyr, Met or Ser resulted in constitutive activity of the rat μ opioid receptor. (Supported by NIH grants DA04745, DA11263)

Mon04

OPIOID RECEPTORS HAVE COMPLEXES

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The dimerization of opioid receptors is a novel mechanism which can influence receptor pharmacology and function. This phenomenon may help explain and consolidate a significant amount of data pointing to functional interactions between opioid receptors types. For example, while most opiates exert their analgesic effects primarily via mu opioid receptors, a number of studies have shown that delta receptor selective drugs can enhance their potency. We have addressed this issue by examining the heterodimerization of mu and delta receptors as a potential mechanism. Here we provide biochemical and pharmacological evidence for a physical interaction between these two classic and clinically important receptors. Co-expression of differentially epitope tagged mu and delta receptors in heterologous cells followed by selective immunoprecipitation results in the isolation of a mu-delta heterodimer. Ligand binding data reveals that mu delta heterodimers display characteristics that are similar to a previously described opioid receptor subtype. Furthermore, a delta selective antagonist is able to enhance the potency and efficacy of a mu agonist. This is observed both in cells heterologously expressing mu and delta receptors and in neuroblastoma cells that endogenously co-express these receptors. This data together with previous finding of kappa delta receptor interactions strengthens the proposition that opioid receptor heterodimers may provide a molecular basis for opioid receptor subtypes. These findings have important clinical ramifications and provide new foundations for more effective therapies.

Mon05

IDENTIFICATION OF A MEMBRANE TARGETING DOMAIN IN THE C-TERMINUS OF THE MU OPIOID RECEPTOR.

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The intracellular C-termini of several receptors have been shown to be involved in the membranal targeting. To investigate structural C-terminal domains involved in the membranal targeting of the mu opioid receptor, we constructed several C-terminal truncation mutants (Trunc 344, Trunc360, and Trunc 386). For immuncytochemical analysis, an epitope tag was added to the N-terminus of all receptor types before stable transfection in HEK 293 cells. As determined by confocal microscopy only the wildtype and the Trunc 386 receptor showed a membranal receptor localization. Sequence analyses revealed a domain between amino acid 373 and 386 which is strictly conserved among all mu opioid receptor types of different species. Fusing this 14 amino acid domain to the shortest truncation receptor (Trunc 344/373-386) led to a membranal localization of this receptor type. In addition all receptor mutants lacking a membranal localization revealed a loss of functional coupling to the adenylate cyclase. Therefore, our data revealed that the C-terminal domain between amino acid 373 and 386 plays important role for the membranal targeting of the rat mu opioid receptor.

Mon06

UP-REGULATION OF A CONSTITUTIVELY ACTIVE MUTANT OF THE RAT μ OPIOID RECEPTOR BY NALOXONE

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Substitution of Asp164(3.49) in the DRY motif of the rat μ opioid receptor with Gln resulted in a constitutively active mutant (D164(3.49)Q), which displayed enhanced basal [^{35}S]GTPγS binding. When transfected into HEK293 or CHO cells, D164Q mutant exhibited little or no [^{3}H]diprenorphine binding. Presence of naloxone in medium greatly enhanced [^{3}H]diprenorphine binding following washout of naloxone. D164Q mutant was stably expressed in CHO cells (CHO-D164Q). The up-regulation by naloxone was dose-dependent (EC_{50} = 2 μM) and time-dependent, reaching a plateau in 72-96 h. An equi-potent dose of naloxone methiodide (a 4^{o}N naloxone analog) caused an up-regulation about 50% of the naloxone effect, suggesting that naloxone acts on extracellular and intracellular sites. Following naloxone removal, its expression level gradually declined to less than 10% after 48 h. Transient co-expression of each of the dominant negative mutants GRK2-K220R, arrestin-2 (319-418), dynamin I-K44A, rab5A-N133I or rab7-N125I in CHO-D164Q cells greatly inhibited the decrease in receptor level following naloxone removal. Pretreatment of CHO-D164Q cells with chloroquine or Proteasome Inhibitor I reduced the down-regulation after naloxone withdrawal. Taken together, these results indicate that the D164Q mutant is constitutively internalized and trafficked through early endosomes, late endosomes into lysosomes and the D164Q mutant receptor appears to be degraded by lysosomes and proteasomes. Thus, naloxone appears to up-regulate the D164Q mutant primarily by inhibiting its constitutive internalization and down-regulation and to stabilize the mutant protein and reduce the denaturation and degradation of the mutant. (NIH grant DA04745, DA11263)

Mon07

MUTATION OF THREONINE 279 IN THE RAT μ OPIOID RECEPTOR DISPLAYS ENHANCED AGONIST AFFINITY AND SUBSTANTIAL UP-REGULATION AFTER NALOXONE PRETREATMENT.

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In many G-protein coupled receptors (GPCRs), point mutations in the C-terminal portion of intracellular loop 3 (IL-3) yield receptors which have been described as constitutively active. Constitutively active receptors display an increased level of agonist-independent activity, enhanced affinity for agonists, and, in some cases, significant levels of up-regulation upon antagonist (or inverse agonist) pretreatment. Based on an alignment of IL-3 of the μ opioid receptor sequence to other GPCRs that have constitutively active mutants, Threonine 279 of the rat μ opioid receptor was selected for mutation. HEK cells stably expressing the mutant receptor display dramatic up-regulation upon naloxone pretreatment relative to the wild type μ opioid receptor. In addition, μ opioid receptor agonists showed enhanced affinity for the mutant receptor in competition binding experiments. Each of these properties has been associated with constitutively active mutants in other systems. The suitability of this mutant as a model for the agonist binding state of the receptor along with potential causes of the observed up-regulation will be presented. Furthermore, structural models of the receptor will be employed to suggest potential structural changes brought about by the mutation.

Mon08

DIFFERENTIAL EXPRESSION OF EIGHT SPLICING VARIANTS ARE DIRECTED BY A NEW PROMOTER OF THE MOUSE MU OPIOID RECEPTOR GENE (MOR-1).

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Using a modified 5'RACE approach, we isolated a novel exon (exon 11), which led to identify three additional exons (exons 12, 13 and 14) and eight novel splice variants (MOR-1G, -1H, -1I, -1J, -1K, -1L, -1M and MOR-1N) of the mouse MOR-1 gene. Exon 11 was mapped to upstream of exon 1 and the others between exons 11 and 2. Exon 11 is the first 5'-end exon for all eight new variants. They all underwent different splicing patterns yielding transcripts for a number of predicted proteins, including the original MOR-1. A single putative transcription start point was determined. Functional characterization of the 5'-flanking region of exon 11 by a SEAP reporter system in several cell lines revealed neuronal-specific promoter activities in the 5' flanking region, which contains a TATA box, two CAAT boxes and several binding sites including C/EBPbeta and AP-1 and cMyc/max. Northern blot analysis with an exon 11 probe shows a diffuse band from 4 to 7 kb. The differential regional distributions of the variant mRNAs, determined by RT-PCR, implied region-specific and/or cell-specific RNA processing. Antisense mapping using two probes targeting exon 11 had different actions spinally and supraspinally, blocking either morphine spinally or M6G supraspinally. Since exon 11 is present in all eight variants, it is not possible to assign a specific variant to these actions, but these findings do suggest that these new variants are pharmacologically relevant. This work was supported by NIDA grants (DA00296) to Y-X.P. and (DA00310) to G.R. and (DA07242, DA02615 and DA00220) to G.W.P.

Mon09

ROLE OF ETS-1 IN THE TRANSCRIPTION REGULATION OF MOUSE δ-OPIOID RECEPTOR

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Three major types of opioid receptors, μ (MOR), δ (DOR), and κ (KOR), have been cloned and characterized. Each opioid receptor exhibits a distinct pharmacological profile as well as a distinct pattern of temporal and spatial expression in the brain, suggesting the critical role of transcription regulatory elements and their associated factors. Previously, Liu et al. reported that an E box and a GC box in the 5`-flanking region of δ-opioid receptor are crucial for DOR promoter activity in NS20Y cells, a DOR-expressing mouse neuronal cell line. Here we report that an Ets-1 binding site immediately upstream of the E box is essential for DOR promoter activity in NS20Y cells. In vitro protein-DNA binding assays and in vivo transient transfection assays indicated that Ets-1 bound to and transactivated DOR promoter via the Ets-1 binding site. As Ets-1 is highly expressed in mouse central nervous system at early fetal stage and in adult lymphocytes, the distinct developmental emergence of δ-opioid receptor in CNS and the expression of δ-opioid receptor in immune system appear to be controlled, at least partially by Ets-1. (Supported by NIDA grants)

Mon10

IDENTIFICATION OF RESIDUES IN THE PUTATIVE SEVENTH TRANSMEMBRANE DOMAIN OF THE HUMAN κ OPIOID RECEPTOR EXPOSED IN THE BINDING POCKET

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Various experimental probes have indicated that binding pockets of GPCRs involve the seven putative transmembrane domains (TMDs) and are accessible from the extracellular medium. (2-Aminoethyl)methanethiosulfonate (MTSEA) is a hydrophilic reagent that reacts specifically with reduced sulfhydryl groups. We previously mapped the residues accessible in the binding-site crevices in the TMD 6 of μ, δ and κ opioid receptors by the substituted cysteine accessibility method (SCAM). In the present study, we applied SCAM to map the residues of the TMD 7 of the κ receptor that are on the water-accessible surface of the binding-site crevices. We mutated to cysteine, one at a time, 25 consecutive residues in the TMD 7 and expressed the mutant receptors in HEK 293 cells. Eight (S7.34C, I7.39C, A7.40C L7.41C, G7.42C, Y7.43C, N7.45C and S7.46) of the 25 mutants were sensitive to MTSEA, and were protected from MTSEA reaction by a reversible antagonist, naloxone. These results indicate that the side chains of these residues are in the water-accessible surface of the binding pocket. The pattern of accessibility of the residues is consistent with the notion that the TMD 7 of the kappa receptor is an (-helix with a kink at the highly conserved P7.50. (supported by NIH grant DA04745)

Mon11

STUDY OF S196A MUTATION OF MU OPIOID RECEPTOR USING KNOCK-IN STRATEGY

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Claude et al have shown that a point mutation in the fourth transmembrane domain of the mu opioid receptor(serine 196 to alanine mutation) confers agonist activity to classical antagonists like naloxone, which is known to have little side-effects(PNAS(1996),93,5715-5719). Delivering the mutant receptor into critical pain-causing locations in patients, combining systemic administration of naloxone, provides a plausible alternative for pain management. To further investigate this possibility and to further characterize the S196A mutation in vivo, a construct was made to introduce this point mutation into mu opioid receptor gene by homologous recombination. SP1 Embroynal Stem cells were transfected with the construct and cells undergone homologous recombination were selected. Microinjection of the ES cells into blastocysts for chimeric mice was carried out. Also, an adenovirus vector expressing mu opioid receptor with the S196A mutation was made for delivering the mutant receptor into mice to study the effects of the mutant mu opioid receptor on pain management. (This work is supported by NIDA grants.)

Mon12

BINDING AND INTERNALIZATION OF FLUORESCENT OPIOID PEPTIDE CONJUGATES: VISUALIZATION IN LIVING CELLS.

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A series of fluorescent dyes were conjugated to dermorphin, deltorphin, TIPP, and endomorphin, in order to visualize the binding and internalization of ligand-receptor complexes in living cells. These new fluorescent peptides were shown to bind and activate μ- or δ-receptors with high affinity and selectivity. Under a confocal microscope the fluorescent peptides stained the membrane surface of living cells either stably expressing μ- or δ-receptors (μ/δ-CHO) or endogenously expressing opioid receptors (NG108-15 and SH-SY5Ycells). Internalization of these opioid agonists was observed by the appearance of intracellular fluorescent puncta. This process was temperature dependent and blocked by naloxone. Internalized agonist accumulated in the perinuclear region in μ/δ-CHO cells, whereas in both neuroblastoma cell lines it was evenly distributed throughout the cytoplasm. Upon conjugation to different fluorophores, the fluorescent dermorphins exhibited differential internalization capabilities. These results suggest that the physico-chemical properties of the dyes might influence the internalization process. In summary, this study demonstrates real-time visualization of binding and internalization of opioid ligands in living cells. These fluorescent peptide conjugates may prove to be valuable tools for studying the dynamics of opioid receptor trafficking.

Mon13

EFFECTS OF OPIOIDS ON CYTOKINETICS IN THE GERMINAL ZONE OF THE EMBRYONIC NEOCORTEX

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Opioid effects on cell division in the ventricular zone of the embryonic day 16-mouse neocortex were examined. [^{3}H]thymidine-labeling (pulse labeled cells) and mitotic indexes were estimated after administration of opioid agonists or the antagonist naloxone to pregnant females. The μ- and κ-agonists DAGO and bremazocine inhibited, whereas the δ-agonist DSLET stimulated cell cycle progression. Exposure to bremazocine affected cell proliferation asymmetrically, influencing cytokinetics only in the left cerebral hemisphere. Naloxone provided a biphasic effect, initially decreasing the duration of the S-phase, followed by the lengthening of the S- and M-phases. Immunohistochemical studies suggested that distinct subpopulations of the ventricular zone cells express μ-, δ-, or κ-opioid receptors. These results suggest that cell division in the ventricular zone of the late embryonic cortex is under tonic control of the endogenous embryonic/maternal opioid system and regulated by multiple opioid receptors and ligands.

Mon14

COMPARISONS OF δ-MEDIATED CONVULSIONS AND ANTINOCICEPTION IN MICE

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Delta opioid ligands cause both δ-mediated antinociception and convulsions in mice. We have hypothesized that a greater ligand efficacy is required for antinociception than for convulsive activity at the δ-receptor. To test this hypothesis and to confirm that the convulsions are δ-mediated both antagonist and knockout studies were performed. Preliminary studies suggest that low doses of the irreversible δ-antagonist naltrindole isothiocyanate (NTII) partially antagonize the antinociceptive properties of BW373U86 without inhibiting the convulsive effects whilst higher doses of NTII can antagonize both. Studies also show differences in the development of tolerance to the antinociceptive and convulsive effects of BW373U86. Together these data suggest that the receptor reserve for δ-mediated convulsive activity may be greater than that for δ-mediated antinociceptive activity. Finally, the convulsive activity of δ-opioid receptor agonists was examined in δ-receptor knockout mice. Both BW373U86 and SNC80 failed to produce any convulsive behaviour in the (-/-) animals demonstrating the absolute involvement of the δ-opioid receptor in this effect.

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Mon15

INTESTINAL OPIOID RECEPTORS (OR): COEXPRESSION OF δ- AND κ-OR IN MYENTERIC NEURONS.

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The intestinal tract is a major site of opiate action and constitutes a important neurobiological model for the study of OR. We characterized OR in myenteric neurons from porcine ileum using immunohistochemical, radioligand binding and functional approaches. Agonists selective for δ- and κ-OR decreased field-stimulated "on" and "off" contractions respectively in circular muscle strips with attached myenteric plexus. μ-OR agonists were ineffective in this assay. Specific binding sites for [^{3}H]naltrindole (Kd = 55 pM; Bmax = 29 fmol/mg protein) and [^{3}H]U-69,593 (Kd = 1.3 nM; Bmax = 16 fmol/mg protein), but not [^{3}H]DAMGO were detected in myenteric neuronal membrane fractions. δ- and κ-OR-like immunoreactivities were colocalized in myenteric neurons using N-terminally-directed anti-OR antisera; no μ-OR immunoreactivity was observed. Future investigations will focus on the significance of δ- and κ-OR interactions. (Funded by NIDA R01 DA-10200).

Mon16

REPEATED KAPPA-OPIOID AGONISTS ALTER UPTAKE AND SYNTHESIS OF DOPAMINE

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To better understand the influence of kappa-opioid agonists on the neurochemical substrates in brain circuits that mediate the behavioral effects of cocaine, rats were treated with daily injections of U-69593 or bremazocine. Kappa-opioid agonists diminish the behavioral activating effects of cocaine. Previously, we have shown that repeated U-69593 injections decrease DA D_2 receptors in the rat brain. In the present study, treatment with the kappa-opioid agonist U-69593 or bremazocine decreased dopamine transporter densities in the caudate putamen. In addition, there was an increase in tyrosine hydroxylase in the caudate putamen of rats treated with either kappa-opioid agonist, compared to those treated with vehicle. Decreased dopamine transporters would initially increase extracellular DA, potentially leading to DA depletion. It is possible that the increase in tyrosine hydroxylase is a compensatory effect. These data suggest that dopaminergic neurotransmission is altered following kappa-opioid agonist treatment, which might explain the decrease in locomotor activity seen in response to cocaine. Supported by DA 11960.

Mon17

BLOCKADE OF HYPERALGESIA TO MORPHINE BY ANTISENSE OLIGODEOXYNUCLEOTIDES TO $G_s\alpha$.

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Morphine, a potent opioid analgesic widely utilized in the clinical setting, exerts its pharmacological effect through activation of specific opioid receptors. In addition to the well characterized inhibition of adenyl cyclase activity, opioids have been described to have stimulatory actions. First, we examined whether we could see an hyperalgesic effect of systemic morphine that could be correlated with a stimulatory effect. We administered morphine subcutaneosuly in doses that ranged from 1 pg/kg to 20 mg/kg, and tested for hyperalgesia 30 min later. We were unable to see hyperalgesia utilizing the tail-flick radiant assay despite increasing the latency time to 7-12 sec. On the other hand, moving to water-immersion assay (49 °C), and based on 7-12 sec latencies, we were able to detect a statistically significant decrease. This decrease was maximal with 300 ng/kg of morphine, and was reversed by the simulataneous administration of 1 mg/kg dose of naltrexone subcutaneously. Moreover, with naltrexone doses of 1pg/kg to 1 ng/kg a statistically significant analgesic effect was even observed. To explore the mechanism underlying the hyperalgesic effect, we utilized an antisense oligodeoxynucleotide paradigm. Downregulation of the $G_s\alpha$ protein resulted in blockade of the hyperalgesic response to morphine. These studies confirm prior suggestions that the stimulatory effect of morphine is mediated through a $G_s\alpha$ coupled mechanism.

Mon18

ACETYLCHOLINE / OPIOID INTERACTIONS IN ACUTE PAIN

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Acetylcholine (ACh) produces antinociceptive effects that are blocked by naloxone. We hypothesize that ACh produces antinociception via the release of endogenous opioids. We here report that ACH-induced spinal antinociception is blocked by naloxone, nor-BNI and atropine, but not by mecamylamine or naltrindole. Thus, the effects of ACh are mediated by both the mu and kappa opioid receptors and muscarinic, but not nicotinic, cholinergic systems. ACh exhibits cross-tolerance to morphine, the kappa agonist CI977, and physostigmine. Conversely, morphine is cross-tolerant to chronic physostigmine. In mu knockout mice, ACh fails to elicit an antinociceptive response in the tailflick test. Quantitation of the direct release of endogenous opioids via spinal cannulation indicates that ACH produces a dose-related 8-fold increase in dynorphin A (1-17) release (blocked by naloxone and atropine). ACh produced a 2-fold increase in leucine-enkephalin release. These results indicate that a functional opioid receptor system is required for ACh-induced antinociception. This work supported by NIDA grants KO2DA00186 and DA05274.

Mon19

RECEPTOR BINDING PROPERTIES OF ADL 8-2698, A POTENT OPIOID RECEPTOR ANTAGONIST

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ADL 8-2698, [[2(*S*)-[[4(*R*)-(3-hydroxyphenyl)-3(*R*), 4-dimethyl-1-piperidinyl] methyl]-1-oxo-3-phenylpropyl] amino] acetic acid, is a potent opioid antagonist that has been shown clinically to reverse opioid slowing of gastrointestinal transit without antagonizing opioid analgesia. ADL 8-2698 is a potent inhibitor of [^{3}H]diprenorphine ([^{3}H]DIP) binding to cloned human opioid receptors with K_i values at μ, κ and δ receptors of 0.43, 99.6, and 9.4 nM, respectively. For comparison, the K_i values for N-methylnaltrexone bromide (MNTX) were 30, 101, and 780 nM and those for naloxone were 3.3, 7.9, and 32 nM, respectively, at these receptors. ADL 8-2698 also inhibited [^{3}H]nociceptin binding to the ORL-1 receptor with a K_i value of 930 nM, while MNTX and naloxone had no effect at 10 μM. Preincubation experiments showed that, like buprenorphine, ADL 8-2698 bound slowly to the μ receptor so that the apparent K_i value at the μ receptor decreased to a minimum of 0.14 nM upon preincubation for 30 min before the initiation of the [^{3}H]DIP binding assay. Preincubation with naloxone or MNTX had no effect on their apparent K_i values. Inhibition of [^{3}H]DIP binding by ADL 8-2698, MNTX or naloxone, but not that by buprenorphine or β-funaltrexamine, was readily reversed by washing. ADL 8-2698 is a potent, moderately μ selective, reversible inhibitor of [^{3}H]DIP binding to opioid receptors.

Mon20

PHARMACOLOGICAL PROFILE OF ADL 8-2698, a PERIPHERALLY REsTRICTED OPIOID ANTAGONIST

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Constipation is a major side effect of opioids used for the treatment of chronic pain. The use of an opioid antagonist that does not penetrate the CNS is a therapeutic approach that could alleviate opioid-induced constipation, yet spare opioid-induced analgesia. ADL 8-2698, [[2(*S*)-[[4(*R*)-(3-hydroxyphenyl)-3(*R*), 4-dimethyl-1-piperidinyl] methyl]-1-oxo-3-phenylpropyl] amino] acetic acid is a potent opioid antagonist that distributes primarily to the GI tract following p.o. or i.v. administration. The goals of these studies were to determine the ability of ADL 8-2698 to block the inhibitory effects of morphine on GI transit (using charcoal meal transit) and secretion (using castor oil-induced diarrhea) and to demonstrate that ADL 8-2698 did not interfere with the central effects of morphine in mice. ADL 8-2698 blocked morphine-induced inhibition of charcoal meal transit with maximal antagonism (95%) at 6 h following 3 mg/kg, p.o. and had an ED_{50} value of 0.46 mg/kg. Similarly, administration of ADL 8-2698 (1 mg/kg, p.o.) resulted in a sustained block (75% antagonism at 24 h) of morphine-induced constipation. In mice rendered acutely dependent to morphine (100 mg/kg, s.c.), ADL 8-2698 at doses up to 30 mg/kg s.c. did not precipitate abstinence-induced jumping. These data demonstrate that ADL 8-2698 blocks the GI effects of morphine without antagonizing effects mediated by central opioid receptors, and support the use of ADL 8-2698 as a palliative agent in patients receiving chronic opiates.

Mon21

INFLUENCE OF APROTININ ON PLASMA LEVEL OF BETA-ENDORPHIN AND SUBSTANCE P DURING WITHDRAWAL IN MORPHINE-DEPENDENT RATS

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Our previous studies show inhibition of opiate withdrawal syndrome by peripheral administration of inhibitor of proteases, aprotinin. The aim of this study was to investigate action of aprotinin on the level of beta-endorphin and substance P in plasma during withdrawal. The experiments were done on morphine-dependent Wistar rats (n=90). It was showed, that significant increase of beta-endorphine and decrease of substance P levels in plasma during withdrawal. Aprotinin in dose 2 mg/kg substantially inhibited withdrawal syndrome. Simultaneously, plasma beta-endorphine was decreased. There was not normalization of substance P level after aprotinin administration. Results show that during opiate withdrawal there is no activation of enzymes, catabolised beta-endorohin. Conversely, pain during withdrawal increase synthesis of beta-endorphine and elevation it plasma level.

Mon22

QUANTITATIVE AUTORADIOGRAPHIC MAPPING OF μ-, δ-, AND κ-OPIOID RECEPTORS IN THE BRAIN OF δ-OPIOID RECEPTOR GENE KNOCKOUT MICE

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Autoradiography of μ-, δ-, and κ-receptors has been carried out in the brain of wild-type, heterozygous and homozygous δ-receptor gene (DOR) knockout mice. [^{3}H]Deltorphin I (7nM), [^{3}H]DAMGO (4nM), [^{3}H]CI-977 (2.5nM) and [^{3}H]bremazocine (2nM in the presence of cold DAMGO and DPDPE) were used to label δ-, μ-, κ_1- and total κ-receptor populations respectively. Non-specific binding was determined in the presence of naloxone. Following short term film exposure no [^{3}H]deltorphin I binding was detected in brains from homozygous mice while there was 50% overall lower binding throughout the brain of heterozygous animals. However, following long periods of exposure (12 weeks) low level [^{3}H]deltorphin I binding could be observed in a number of brain areas, these regions significantly correlated with those demonstrating moderate to high levels of μ-receptor expression. Both μ- and κ_1-receptor populations demonstrated a significant overall decrease in binding in homozygous DOR-deficient mice compared to wild-type animals. In contrast, overall levels of [^{3}H]bremazocine binding were significantly higher in homozygous than wild-type animals. Our findings suggest that disruption of the DOR gene results in loss of expression of a δ-receptor population. Furthermore, our results indicate that [^{3}H]deltorphin I can exhibit break-through labelling of μ-receptors in the absence of a δ-receptor population. Findings also suggest that [^{3}H]CI-977 and [^{3}H]bremazocine label either different receptor populations or different receptor states. Supported by EC grant BMH4-CT96-0510.

Mon23

ANABOLIC-ANDROGENIC STEROIDS – EFFECTS ON OPIOID RECEPTORS AND BEHAVIOR

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Anabolic-androgenic steroids (AAS) have been abused by elite athletes and bodybuilders for decades but has in recent years also been connected to several horrifying crimes and acts of violence. The adverse physiological effects of AAS are well established. However, also psychological side effects such as depression and aggression have been reported. It has furthermore been reported that persons not connected to sports uses AAS to increase self-esteem and aggressiveness. Aggressive behavior is controlled by the mesolimbic system such as amygdala, PAG, hypothalamus and striatum, which are important structures in mediating this behavior. Furthermore enkephalin and substance P (SP) have been shown to be involved in the aggression pathways. We have recently shown that AAS affects the level of opioid peptides as well as the SP in the mesolimbic system. In the present study the effect of chronic treatment with nandrolone decanoate, an AAS, on the expression of opioid receptors and NK1 were examined in the brain of male rats. The rats were treated with a supraphysiological dose (15 mg/kg/day) of nandrolone or vehicle by i.m. injections during 14 days. We have also studied defensive aggression in the rats treated with AAS. Results indicated that steroid-induced effects were observable both in behavior and at the receptor level. The relevance of this will be discussed.

Mon24

CONTRIBUTION OF SPINAL NOVEL μ-OPIOID RECEPTOR TO ENDOMORPHIN-2-INDUCED ANTINOCICEPTION

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Endomorphin-1 (EM-1) and -2 (EM-2) are two tetrapeptides recently isolated from the bovine as well as human brains. Both endomorphins can produce a potent and prolonged antinociceptive activity with a high affinity and selectivity for the μ-opioid receptor antagonist β-funaltrexamine (β-FNA). In the present study, the role of μ-opioid receptor subtypes in the antinociceptive effects of intrathecally (i.t.) injected EMs was examined with a novel antagonist selective for μ-opioid receptor subtypes, 3-methoxynaltrexone (3-MNT) in the mouse paw-withdrawal test. Pretreatment with β-FNA result in a significant reduction of the antinociceptive effects of EM-1 and EM-2. Antinociception induced by i.t. injection of EM-1 was not reversed by pretreatment with 3-MNT, whereas EM-2-induced antinociception was antagonized significantly by 3-MNT. The results indicate that EM-1 and EM-2 may produce antinociception through the distinct subtypes of μ-opioid receptors.

Mon25

NORBUPRENORPHINE IS A POTENT OPIOID AGONIST

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Buprenorphine(BUP) is an oripavine analgesic that is beneficial in the maintenance treatment of opiate-dependent individuals. Although BUP has been studied extensively, relatively little is known about norBUP, a major metabolite of BUP. We now describe the binding of norBUP to opioid receptors and its pharmacological effects in opioid receptor-mediated [^{35}S]GTPγS binding and in the mouse (0.6% acetic acid) writhing test. CHO cells stably transfected with the μ, δ or κ opioid receptors were used. NorBUP exhibited high affinities for μ, δ and κ opioid receptors with K_i values in inhibiting [^{3}H]diprenorphine binding of 0.07, 3.14 and 0.91 nM, respectively. Corresponding values for BUP were 0.08, 0.42 and 0.11 nM, respectively, while (+)-BUP was inactive. In the [^{35}S]GTPγS binding assay, norBUP was a partial agonist at the μ and κ receptors and a full agonist at the δ receptor. EC_{50} values were 1.5, 7.2, and 30.4 nM, respectively; maximal responses were 84%, 56% and 96% of those of standard full agonists. BUP was a partial agonist at the μ and κ receptors with EC_{50} values of 0.08 and 0.04 nM and maximal responses of 44% and 12%, respectively, and was inactive at the δ receptor. In the writhing test, BUP and norBUP both suppressed writhing in an efficacious and dose-dependent manner, giving A_{50} values of 0.067 and 0.21 mg/kg s.c., respectively. These results highlight the similarities and differences between BUP and norBUP, each of which may influence the pharmacological profile of BUP. (DA04745, DA11263 and DA07237).

Mon26

POSSIBLE INVOLVEMENT OF MORPHINE-INSENSITIVE MU_1-OPIOID RECEPTORS IN THE FENTANYL-INDUCED ANTINOCICEPTION.

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The present study was designed to investigate the mechanisms of fentanyl (FEN)- and morphine (MRP)-induced antinociception. In the tail flick test, either FEN or MRP produced mu-antagonist-reversible antinociception in ddY and C57BL/6 mice after either s.c., i.c.v. or i.t. injection. On the contrary, either FEN or MRP failed to produce antinociception in mu_1-deficient CXBK mice, indicating that antinociception induced by FEN and MRP was mediated predominantly through mu_1-opioid receptors at both supraspinal and spinal levels. We have already reported that ATP-sensitive potassium (K+) channel appears to play an important role in the MRP-induced antinociception. In the present study, we investigated the effects of glibenclamide, an ATP-sensitive K+ channel blocker, on the FEN- or MRP-induced antinociception. The MRP-induced antinociception was significantly attenuated by pretreatment with either i.c.v. or i.t. glibenclamide, whereas antinocicepion produced by FEN was not affected by glibenclamide, indicating the different mechanism of FEN-induced antinociception. Furthermore, i.c.v. glibenclamide did not affect i.c.v. MRP-induced inhibition of gastrointestinal transit which is thought to be mediated by mu_2-opioid receptor. These findings suggest that ATP-sensitive K+ channel may not be involved in mu_2-opioid receptor-mediated pharmacological actions. In conclusion, these present data indicate that FEN-induced antinociception may be mediated through MRP-insensitive mu_1-opioid receptor subtypes at both supraspinal and spinal levels, raising the possibility of mu_1-receptor subtypes.

Mon27

THE EFFECT OF P-GLYCOPROTEIN ON OPIATE TRANSPORT AND ANALGESIA.

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Interactions between the brain and the body are complex. While the peripheral nervous system provides important pathways for communication, evidence now indicates that the brain is capable of releasing important peripherally acting neurochemicals. Intracerebroventricular (i.c.v.) administration of ^{125}I-[D-Pen2,D-Pen5]enkephalin, ^{125}I-β-endorphin or ^{125}I-Morphine leads to their rapid appearance in blood. The transport system is saturable, with the efflux of ^{125}I-β-endorphin and ^{125}I-Morphine competed by _-endorphin and morphine, respectively, in a dose-dependent manner. Immunohistochemically, high levels of Pgp1 were observed in the choroid plexus. Antisense oligodeoxynucleotides downregulating Pgp1, dramatically reduced the efflux of iodinated compounds from the brain to the circulation and altered the analgesic activity of β-endorphin and morphine. These findings demonstrate: 1) the brain's ability to secrete compounds to the periphery via the Pgp1 transporter, illustrating a potentially important mind/body communication pathway and 2) the Pgp1 transporter's role in the production of β-endorphin and morphine analgesia.

Mon28

NONOPIOID MOTOR EFFECTS OF DELTORPHIN ANALOGS

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met-deltorphin analogues bearing D-Ile, D-alle, D-Val and D-nLeu in the second position displayed lower δ-affinity and δ/μ selectivity, in receptor binding experiments, and lower efficacy, in stimulating [^{35}S]GTPγS binding to rat brain membranes, than *ala*-deltorphin-I and II. When given i.c.v. in rats, *met*-DELT analogues induced a dose-dependent analgesia (ED_{50}=8-20 μg/rat) antagonized by naloxone (0.1 mg/kg, s.c.) but unaffected by naltrindole (3 mg/kg, s.c.). High doses (10-50 μg/rat) always produced characteristic motor disfunction such as hind-limb jerking, barrel rolling, circling, ataxia and unusual contorted posture lasting 20-60 min. These motor effecs were not antagonized by s.c. preadministration of naloxone (3mg/kg), naltrexone (10mg/kg) and naltrindole (3mg/kg). The competitive and noncompetitive NMDA antagonists CPP (2 nmol/rat),7-chlorokynurenic acid (80nmol/rat), ifenprodil (4mg/kg), ketamine (10mg/kg), MK-801 (20nmol/rat), the D-antagonist aloperidole (2 mg/kg), the anticonvulsant drug phenytoin (20mg/kg),the s_1-receptor antagonist BD-1047 (50nmol/rat) were inefficacious. On the contrary, both barrel rotations and motor disfunctions were completely blocked by the noncompetitive NMDA antagonist dextrorphan (5 nmol/rat) and by the s_1-receptor agonist (+)-SK&F 10047 (4 mg/kg). Such a nonopioid motor effects are structure dependent: the analogues of *ala*-deltorphin bearing a His residue in position four (like all the *met*-DELT analogs) produced similar syndrome.

Mon29

CHARACTERIZATION OF [^{3}H]ENDOMORPHIN 1 BINDING IN RAT BRAIN MEMBRANES

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Previous results have suggested that endomorphin 1 is a highly selective endogenous mu-opioid receptor (MOR) agonist. In this study we present the characterization of tritiated endomorphin 1 binding to crude rat brain membrane preparation. [^{3}H]endomorphin 1 was prepared by catalytic dehalo-tritiation of its diiodinated precursor (specific activity: 41 Ci/mmol). The binding was saturable, stereo specific and of high affinity (K_d: 1 nM); non-specific binding was determined by using 10^{-5} M naloxone. The maximum number of binding sites (B_{max}) was: 188 fmol/mg protein. In competitive binding experiments [^{3}H]endomorphin 1 was displaced by highly selective MOR ligands DAMGO, endomorphin 2 and levorphanol (K_i: 0.7 nM, 2 nM and 0.25 nM; respectively) while the delta selective Ile^{5,6}-deltorphin 2 and TIPP and the kappa selective dynorphin (1-11) and MERF were much less potent in inhibiting the binding. The K_i value obtained in homologous displacement experiment with unlabelled endomorphin 1 (1 nM) was identical with the K_d value obtained in saturation binding studies. In conclusion, these results show that endomorphin 1 binds to rat brain membrane in a naloxone sensitive manner with nanomolar affinity, providing further evidence for its MOR specificity. The use of [^{3}H]endomorphin 1 can promote the further understanding of the opioid system at the molecular level. Supported by OTKA T022104 T03086 and János Bolyai Fellowship (I.L.).

Mon30

PHARMACOLOGICAL CHARACTERIZATION OF SUPER DALDA, A POTENT μ-OPIOID ANALGESIC

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The dermorphin-derived peptide [Dmt^1]DALDA (H-Dmt-D-Arg-Phe-Lys-NH_2), also known as SUPER DALDA, has high μ-opioid receptor affinity and selectivity, as shown by competition binding assays where it afforded K_i values of 0.05, 0.27, 116 and 21.2 nM for binding to μ_1, μ_2, δ and κ_1 opioid receptors respectively. SUPER DALDA lowered κ_3 receptor binding in a biphasic manner, with K_i values of 0.69 and 187 nM. In the mouse radiant heat tail-flick assay SUPER DALDA produced analgesia when administered intradermally into the tail (ED_{50} 7.6 μg), implying peripheral activity. Given systemically, the peptide produced profound analgesia being more than 100-fold more potent than morphine. This effect was inhibited by prior administration of naloxone given systemically or supraspinally. SUPER DALDA is even more effective upon spinal administration, with an ED_{50} of ~0.1 ng which is 3000-fold more potent than morphine. Antisense mapping targeting MOR-1 exons suggests that SUPER DALDA mediates analgesia *via* a μ-receptor mechanism of action distinct from that of morphine. Thus, SUPER DALDA is an interesting and extraordinarily potent, systemically active peptide analgesic, therefore providing alternative and novel approaches to the design of clinically useful therapeutics.

Mon31

THE ACTION OF DELTA ANALGESICS IN DOR-1 AND ENKEPHALIN NULL MICE

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Our lab has previously found that i.c.v DPDPE and Deltorphin II analgesia are retained in DOR-1 knockout mice, while the activity of BW373U86 (a non-peptidic delta selective drug) is increased after both i.c.v. and s.c administration, suggesting that a second delta-like analgesic system has become unmasked. We have here used the tail-flick assay of nociception in both DOR-1 null and preproenkephalin (ENK) null mice to study the action of two closely related delta selective drugs BW373U86 and SNC-80. Like wild type mice, ENK null mice do not show analgesia following s.c. injection of BW373U86 at 30 mg/kg. This suggests that disruption of the delta receptor specifically, and not its endogenous ligand, is necessary to cause an up-regulation BW373U86 sensitivity. Unlike BW373U86, SNC-80 (a drug with greater delta specificity) does not produce analgesia following s.c. administration in the DOR-1 null mice, while it produces significant analgesia in wild type mice. These data would suggest that SNC-80 acts entirely through the DOR-1 gene product and does not activate the second delta-like system, which can be mobilized by BW373U86. Taken as a whole these data indicate that, although closely related structurally, BW373U86 and SNC-80 possess distinct pharmacological properties. Disruption of the DOR-1 gene has been shown to ablate tolerance to morphine and DPDPE. To analyze the tolerance liability of BW373U86 in this strain, mice were subjected to a chronic drug regimen consisting of once daily s.c. injections of BW373U86 at 30 mg/kg. In DOR-1 knockouts, complete tolerance to BW373U86 occurs by the second day of treatment and persists for at least three weeks. These results would indicate that DOR-1 is not required for BW373U86 tolerance it is for morphine and DPDPE tolerance.

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Mon32

3-D ISOBOLOGRAPHIC ANALYSIS OF INTRATHECAL MU OPIOID, ALPHA-2 ADRENOCEPTOR AND 5-HT RECEPTOR MEDIATED ANALGESIA.

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Stimulation of alpha-2 and 5-HT receptors produce analgesia that is additive or synergistic to morphine-induced analgesia. When injected spinally, morphine produces analgesia primarily through μ_2 receptors, NE through alpha-2 receptors and 5-HT through 5-HT3 receptors. Accordingly, we examined the three-dimensional interaction of analgesia produced by activation of these receptor subtypes. Morphine, NE and 5-HT, injected i.t. each produced tail-flick analgesia dose-dependently in mice. NE was synergistic with morphine, whereas 5-HT was additive. The combination of NE and 5-HT was additive. The analgesia produced by isobolic combinations of i.t. morphine, NE and 5-HT showed no further synergism of analgesia beyond that seen with 2-dimensional interactions. Similarly, the μ_2 agonist Tyr-W-MIF-1, the alpha-2 agonist clonidine and the 5-HT3 agonist 2-methyl-5-HT each produced tail-flick analgesia dose-dependently. Analgesia produced by i.t. Tyr-W-MIF-1was additive with i.t. 2-methyl-5-HT. However, Tyr-W-MIF-1 and clonidine were synergistic, and 2-methyl-5-HT and clonidine were additive. Concurrent administration of these three drugs produced analgesia that was greater than predicted by the two-dimensional interactions. Because constipation is a common complaint among patients treated with morphine, we assessed the effects of combinations of morphine, NE and 5-HT on GI transit. Whereas morphine alone inhibited GI transit, the administration of combinations that produced analgesic synergy produced no inhibition of GI transit.

Mon33

THE ANTINOCICEPTIVE EFFECT OF MIRTAZAPINE.

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The antinociceptive effects of the noradrenergic and specific serotonergic antidepressant (NaSSA) drug mirtazapine and its interaction with opioid receptor subtypes were evaluated in the hotplate. Mirtazapine elicited an antinociceptive effect in a biphasic manner. This effect of mirtazapine was antagonized by naloxone, implying a possible opioid mechanism of action. In addition mirtazapine induced antinociceptive effect was also antagonized by the serotonin antagonist metergoline and by the noradrenergic antagonist yohimbine, implying the involvement of serotonergic and noradrenergic mechanisms as well. These results suggest a potential use of mirtazapine in the management of some pain syndromes. However, further research is needed in order to establish both the exact clinical indications and the effective doses of mirtazapine when prescribed for pain.

Mon34

(2S,3R)TMT-L-TIC-OH IS A POTENT AND SELECTIVE ANTAGONIST AT THE δ OPIOID RECEPTOR IN MICE

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We are investigating the antinociceptive and G-protein stimulating effects of (2S,3R)TMT-L-Tic-OH (TMT) in mice. Based on prior evidence of this substance having antagonist properties, we performed mouse tail flick assays following i.c.v. deltorphin II (Del II) in mice pretreated with either i.c.v. TMT or vehicle. TMT was prepared in 5 µl distilled water and injected 10 minutes prior to injection of Del II. Del II produced antinociception in vehicle treated mice with an A_{50} value of 9.95 nmol after 20 minutes. 30 nmol TMT alone had no effect, whereas 20 and 30 nmol TMT dose-dependently blocked 30 nmol Del II-mediated antinociception 30 minutes after Del II administration. We also examined stimulation of [^{35}S]GTPγS binding in mouse brain membranes by SNC80 and DAMGO in the presence and absence of TMT. Whereas TMT showed no response, SNC80 and DAMGO had EC50's in this system of 246 ± 20 nM and 1457 ± 628 nM, respectively. TMT dose-dependently shifted the SNC80 curve to the right with a K_e value of 3.61 ± 0.71 nM, whereas the DAMGO curve was not significantly shifted at 3000 nM TMT. These findings suggest that TMT is a high affinity and selective antagonist at the δ opioid receptor in mice. (Supported in part by the ADCRC and NIDA grants.)

Mon35

DIFFERENTIAL INVOLVEMENT OF SPINAL κ-OPIOID RECEPTORS IN ENDOMORPHIN-1- AND -2-INDUCED ANTINOCICEPTION

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Two highly selective µ-opioid receptor agonists, endomorphin (ME)-1 and ME-2, have been isolated from bovine brain and identified. We determined the antinociceptive effects of the endomorphins at the spinal level in the paw withdrawal test in mice. Antinocieption induced by EM-1 was blocked by pretreatment with β-funaltrexamine (β-FNA; 40mg/kg, s.c.) but not naloxonazine (NLZ; 35mg/kg, s.c.), nor-binartolphimine (nor-BNI; 10mg/kg s.c.) or naltrindole (4mg/kg, s.c.). In contrast, ME-2-induced antinociception was blocked by pretreatment with β-FNA, NLZ, nor-BNI or an antiserum against dynorphin A(1-17) but not naltrindole or an antiserum against dynorphin B(1-13). The results indicate that EM-2 in the spinal cord may stimulate µ-opioid receptors sensitive to NLZ, which subsequently induce the release of dynorphins that act on κ-opioid receptors to produce antinocieption.

Mon36

DOWN REGULATION OF δ-OPIOID RECEPTOR IN NG108-15 CELLS USING Nt-ANTIBODY

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In order to observe significant changes in receptor phosphorylation, we have overexpressed the δ–Opioid receptor in NG108-15 cells using mDOR DNA. These cells are desensitized after 1 h. of exposure to DPDPE (2-D-Penicillamine, 5-D-Penicillamine). After desensitization the receptor displayed approximately 50% inhibition in binding of [^{3}H] DPDPE, with a simultaneous increase in the phosphorylation of the 58 kDa receptor protein. Increase in phosphorylation was significantly decreased in the presence of 100nm PD98059 an inhibitor of MAP kinase. Binding data from saturation and competition experiments using [^{3}H] DPDPE showed that Nt-Antibody competes with the ligand for binding to the δ-Opioid receptor.Nt-Antibody is used to localize the receptor in NG108-15 cells after desensitization .

Mon37

PRESYNAPTIC MODULATION BY OPIOID RECEPTORS ON EXCITATORY AND INHIBITORY TRANSMISSION TO RAT SUBTHALAMIC NEURONS

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Whole-cell recordings were made from neurons *in vitro* to examine opioid modulation of synaptic transmission in rat subthalamic nucleus (STN). [Met]-enkephalin (10 microM) reduced $GABA_A$ IPSCs by 38 ± 4%, and glutamate EPSCs by 17 ± 4%. The mu-receptor agonist [D-Ala^2, N-$MePhe^4$, Gly^5-ol]enkephalin (DAMGO, 1 microM) inhibited IPSCs and EPSCs by 36 ± 6% and 17 ± 4%, respectively. The delta-receptor agonist [D-Pen^2, D-Pen^5]enkephalin (DPDPE, 1 microM) inhibited IPSCs by 19 ± 4% and EPSCs by 8 ± 3%. These effects were blocked by selective mu- and delta-receptor antagonists. Enkephalin, DAMGO and DPDPE increased the paired-pulse ratio of IPSCs or EPSCs. The kappa-receptor agonist U-69693 slightly inhibited IPSCs. Orphan opioid agonist OFQ had no effect. These result suggest that presynaptic modulation of inhibitory inputs to the STN mainly occurs through mu- and delta-opioid receptors while presynaptic modulation of excitatory input to the STN mainly occurs through mu-opioid receptor.

Mon38

LOW DOSES OF MORPHINE ELICIT ACUTE THERMAL HYPERALGESIA IN NORMAL MICE

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Cotreatment of mouse DRG neurons in culture with pM naloxone or naltrexone (NTX) selectively antagonizes excitatory, Gs-coupled opioid receptor-mediated effects elicited by fM-nM morphine and unmasks potent opioid inhibitory, Gi/Go-coupled effects (C & S, PNAS, '95). This predicts that low-dose morphine may be hyperalgesic in vivo. To test this hypothesis we used hot water-immersion tail-flick antinociceptive assays at 52°C in normal mice. Low doses of morphine (μg/kg) did, in fact, elicit acute *hyperalgesic* effects, manifested by *shortening* of the opioid-induced tail-flick latency that lasted for hours after drug treatment. Ultra-low-dose NTX not only blocked the opioid-induced hyperalgesia but also unmasked substantial analgesic effects of this low dose of morphine that did not show tolerance after 3 daily co-injections. The data suggest that doses of morphine far below those currently required for clinical treatment of pain may become effective when opioid hyperalgesic effects are blocked by co-administration of appropriately low doses of NTX, thereby markedly attenuating morphine tolerance, dependence and other aversive side effects (C & S, TiPS, '98, PAIN '00).

Mon39

MU, KAPPA, AND DELTA OPIOID RADIOLIGAND BINDING IN AMPHIBIAN BRAIN

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We are interested in the mechanisms of opioid analgesia in non-mammalian vertebrates to determine the evolution of opioid receptors. Recent behavioral and binding data show that a single opioid receptor type may be mediating the observed analgesia in the amphibian, *Rana pipiens*. This conclusion is based on 1) spinal administration of μ, κ, or δ-selective opioid agonists and antagonists did not show selectivity with each type of antagonist blocked the analgesia mediated by each type of agonist, and 2) labeled naloxone studies in brain and spinal cord show a single opioid binding site which has the same competition constants for the three selective opioid antagonists. Present studies characterized the affinity and density of binding sites after incubation of brain homogenates with tritiated DAMGO (μ),U69593 (κ), or DPDPE (δ). The results show a similar affinity and density of opioid binding sites for each labeled agonist. Each selective agonist is displaced most effectively by the same type of agonist or antagonist. We propose that the amphibian receptor (termed the unireceptor) has three selective sites on the extracellular loops for each of the opioid agonists. Support by NIH grant NIDA 12448 to CWS.

Mon40

ANTI-PRURITIC EFFECT OF κ OPIOID RECEPTOR AGONIST TRK-820.

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The pathogenesis of uremic, cholestatic, or atopic pruritus is uncertain, but recent findings suggest that endogeneous opioid peptides relate to these intractable pruritus and μ opioid antagonists, such as naloxone, suppress an itch sensation in those patients. Their use is, however, limited by opioid withdrawal reactions or adverse effects.

It has been reported that activation of the κ opioid receptor antagonizes various μ mediated actions. We have evaluated the efficacy of a selecitve κ opioid receptor agonist TRK-820 in mouse pruritus model with 'scratching behavior' induced by intracisternal injection of morphine. Morphine (0.1-0.3 nmol/mouse, i.c.) produced a dose-dependent increase in scratching behavior, and this behavior was 5-10 min in peak and appeared intermittently for at least 20 min after the injection. But, a higher dose of morphine (1-10 nmol) showed less or no increase in scratching behavior and an apparent increase in locomotor activity. The number of morphine (0.3 nmol/mouse)-induced scratching behavior was reduced by TRK-820 dose-dependently, accompanied by no apparent gross behavior. On the other hand, ketotifen, an anti-histamine drug, had poor effect in this model. These results suggest that TRK-820 has a potential therapeutic value in relieving severe pruritus, which are less sensitive to an anti-histamine drug. In addition, morphine-induced scratching was shown to be useful model in evaluating intractable pruritus.

Mon41

EXPRESSION OF NOVEL GUINEA PIG UGTs: EFFECT OF MORPHINE ON REGULATION IN NEAR-TERM PLACENTA. D.P. Andrews, S.R. Nagalla, G.D. Olsen, S.A. Smith. Departments of Pediatrics, and Physiology and Pharmacology, Oregon Health Sciences University, Portland, OR.

Uridine diphosphate glucuronosyltransferases (UGTs) are a family of enzymes that act to conjugate a glucose moiety to a multitude of endogenous (steroid hormones, thyroxine, retinoic acid, etc.) and exogenous substrates, such as morphine. Our laboratory has cloned 4 novel UGTs from the guinea pig. This study was undertaken to determine if these UGTs are expressed in the placenta and whether exposure to chronic intermittent morphine from dosing of the pregnant dam would affect the expression. We also examined the effect of fetal gender on placental expression of the UGTs. RNA from morphine-treated and saline-treated near-term guinea pig placental tissue was analyzed by semi-quantitative RT-PCR for UGT expression. UGT2A3, UGT2B31, and UGT2B32 expression was present in placental tissue. UGT1A07 was not detected in any placental RNA. Morphine exposure consistently increased expression of UGT2A3 in the male placenta, but decreased expression in the female placenta. UGT2B31 and UGT2B32 did not show consistent regulation with morphine exposure or fetal gender. Regulation of UGT expression may affect the metabolism of steroid hormones in the placenta and result in poor fetal growth. (Supported by KO8 DA00295).

Mon42

INCREASED WEIGHT AND ADIPOSITY IN MICE LACKING β-ENDORPHIN.

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Opioids have been shown to regulate food intake and preference as well as the release of hormones involved in weight homeostasis. To determine the role of the endogenous opioid β-endorphin in weight homeostasis we investigated feeding, metabolism, and hormone responses in β-endorphin deficient mice. Growth curves showed that male mice lacking β-endorphin are 15% heavier than male wild type mice. In contrast, female mice lacking β-endorphin were not significantly heavier than wild type female mice. Analysis of fat stores showed that both the peri-renal and gonadal white fat stores of male but not female β-endorphin knockout mice were 2-fold heavier than wild type mice. Histological annalysis showed the increase in adiposity is due to an increase in the size of adipocytes rather than an increase in number. Preliminary results suggest that the increase in weight is due to increased food intake rather than decreased basal metabolic rate. Transgenic rescue experiments showed that re-expression of β-endorphin to both the CNS and pituitary but not the pituitary alone was sufficient to fully rescue both the weight and fat phenotype. These results suggest a critical role for CNS β-endorphin expression from the proopiomelanocortin gene in normal weight homeostasis and adipose mass regulation. Supported by: NIH grants DK55807 & DK10082.

Mon43

PROPRANOLOL DECREASES DEGRADATION OF MET-ENKEPHALIN-ARG-PHE ACROSS THE HEART. **B. A. Barron, E. B. Pearlman. Dept Integrative Physiology, Cardiovascular Research Institute, UNT Health Science Center, Fort Worth, TX.**

Catecholamines may decrease the degradation of enkephalins in plasma. To test whether this is a receptor mediated process, spillover was measured across the cardiac vascular bed. ^{3}H-met-enkephalin-arg-phe (MERF) was infused to steady state in anesthetized, instrumented dogs and blood was sampled from the arterial and venous drainage across the heart. Propranolol (n=8) or saline (n=10) was given iv after steady state samples were taken and then either saline or isoproterenol (n=6) was infused for five minutes and another sample was taken. Blood was processed to separate intact from degraded MERF. The venous-arterial difference (V-Adif) of intact MERF was multiplied by the coronary blood flow to give an estimate of spillover. Propranolol was used to enable infusion of catecholamines without changing coronary blood flow which can change spillover. Coronary flow was not different between any group. The V-Adif was negative in all groups at steady state. In the presence of propranolol the V-Adif became less negative to positive in some animals. A negative V-Adif indicates either uptake or degradation across the vascular bed. A positive V-Adif indicates a net release from the tissue. Propranolol plus isoproterenol was not different from control. Propranolol may be blocking the effect of endogenous catecholamines or may have a non-specific drug effect to protect MERF from enzymatic degradation or uptake by the tissue.

Mon44

DELTA OPIOID RECEPTOR ANTAGONIST-MEDIATED CELL GROWTH AND APOPTOSIS OF HUMAN LUNG CANCER CELLS.

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It has been reported that opioids can modulate cell proliferation and survival. We decided to use small cell lung cancer cells as a model to address the following two fundamental questions: (1) Are opioids critical in proliferation of opioid receptor-expressing cancer cells? (2) How do opioids modulate cell growth and survival of cancer cells? Here we demonstrate for the first time that delta opioid receptor-specific antagonist naltrindole (NTI) potently blocks the cell proliferation and induces the apoptosis of small cell lung cancer cells. NTI blocks phosphorylation at Serine 473 of protein kinase B (PKB or Akt) while the non-specific opioid receptor agonist etorphine enhances the phosphorylation in cells. Our preliminary results indicate that NTI may mediate cell growth and survival by blocking the receptor-mediated activation of the PKB-regulated survival signaling. More importantly, the results suggest that delta opioid receptor-specific antagonists may serve as a novel type of potential anticancer agents (Supported by NIDA grants).

Mon45

DIFFERENTIAL CARDIORESPIRATORY AND ANALGESIC EFFECTS OF ENDOMORPHINS 1 & 2.

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The novel endogenous mu-opioid receptor (MOR) agonists endomorphin 1 (EM1) and 2 (EM2) were tested for their cardiorespiratory effects in unanesthetized rats. Intravenous injection of EM1 and EM 2 (5-9,600 nmol/kg each), DAMGO (5-2,400 nmol/kg) and morphine (30-9600 nmol/kg) to unanesthetized rats induced analgesia at 900 nmol/kg for the peptide mu-opioid receptor (MOR) agonists and 1725 nmol/kg for morphine. EM1, EM2 and DAMGO elicited biphasic minute ventilation (V_E) responses characterized by marked, short-lived V_E depressions (4-6 sec) followed by a more sustained V_E increase (10-12 min). Morphine produced a V_E depression, but no subsequent V_E increase. However, V_E depression by EM1 occurred only at higher doses, and the V_E stimulatory effect was greater than that of EM2 or DAMGO. EM2 and DAMGO induced a transient bradycardia and hypotension (>1SD from control), while EM1 induced bradycardia, but no decrease in BP at doses up to 9,600 nmol/kg. The decreases in V_E observed after EM1,EM2 and DAMGO were blocked by naloxone HCl, but not by naloxone-methiodide, indicating a central action. The bradycardic and hypotensive responses were blocked by Nlx and MeNlx. We conclude that MOR-selective compounds vary in their cardiorespiratory response characteristics which could be linked to differential cellular actions. The results support the concept that the analgesic and respiratory effects of mu-agonists can be dissociated and that EM1-like compounds could provide the basis for novel, safer analgesics.

Mon46

SUPER DALDA ENHANCES ADRENERGIC RESPONSES IN THE DOG.

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Szeto et. al reported that systemic infusion of the highly selective mu agonist, super DALDA increased blood pressure when administered in sheep. The current studies were conducted to test the hypothesis that super DALDA alters cardiovascular responses to autonomic activation in the dog. Several methods were employed to activate the autonomic systems responsible for the control of heart rate and blood pressure. These methods included the infusion of norepinephrine into the sinoatrial (SA) node by microdialysis, the direct electrical stimulation of sympathetic and vagal nerves and the reflex activation (bilateral carotid occlusion) of the sympathetic input to the heart. Each challenge was conducted in the presence and absence of super DALDA. Blood pressure and heart rate were monitored throughout. ANOVA and Tukeys test were used to analyze the data. Super DALDA had no effect on the bradycardia (n = 5) during efferent vagal stimulation. Blood pressure was significantly higher when carotid occlusion and super DALDA were combined; however, heart rate was unaltered. In contrast, heart rate was significantly higher when super DALDA was combined with exogenous norepinephrine, administered into the SA node via microdialysis. There was no apparent effect of super DALDA on heart rate or blood pressure during direct sympathetic nerve stimulation. Facilitation of tachycardia during sympathetic nerve stimulation may have been obscured by the large variability and limited number of responses obtained thus far. The current results suggest that super DALDA may enhance both the pressor and heart rate responses to norepinephrine in the canine model. Additional subjects will be required to verify these findings and determine the underlying mechanism.

Mon47

VERIFICATION OF A μ OPIOID RECEPTOR MODEL BY Zn2+-BINDING SITE ENGINEERING

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We have constructed three-dimensional computational models of the μ, δ, and κ opioid receptor transmembrane domains by distance geometry with H-bonding constraints [*Biophys J.* **75**, 612 (1998)]. Comparison of our model to an EM-based model [*J. Mol. Biol*. **272**, 144 (1997)], showed that there are differences in the alignment of various transmembrane helices. To clarify the vertical alignment of these helices, we constructed potential Zn^{2+}-binding centers by mutating residues located near the proposed binding pocket of the μ opioid receptor to Cys or His, making use of a native histidine as one of the Zn^{2+} coordination sites. The demonstration of Zn^{2+} binding, as evidenced by reduced ligand binding in the presence of Zn^{2+}, indicates that those residues forming the Zn^{2+}-binding center are in close proximity. By comparing the Zn^{2+} binding ability of engineered binding centers composed of residues located at varying positions along the helices, the relative vertical alignment of these helices can be determined. Single point mutations of residues around the binding pocket also test our proposed model of ligand docking inside the opioid receptor.

Mon48

SYNERGISM OF MORPHINE AND HIV-1 TAT PROTEIN PROMOTE TOXICITY IN MURINE STRIATAL NEURONS

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Injecting drug users (IDUs) such as heroin abusers are at high risk for contracting human immunodeficiency virus (HIV). Interestingly, HIV targets the striatum, a region rich in opioid receptors. Novel evidence suggests that HIV can release neurotoxic products, which may partially account for an increased incidence of HIV encephalitis in HIV infected IDUs. In the current studies, the potential toxic interaction of opiates (morphine and naloxone) and HIV neurotoxin (Tat) was examined in murine striatal neurons. Phenotypically, striatal neurons were μ, κ, and to a lesser extent δ immunopositive. Following exposure to opioids and Tat, viability was assessed separately by time-lapse photography (16 hr post-treatment) and ethidium monoazide exclusion (24 hr post-treatment). Synergistic toxicity between morphine (1 μM) and Tat (100 nM) was apparent after 16 hours, causing a 55.6% reduction in viable neurons compared to untreated groups (P<0.05). At 24 hr, morphine (10 nM, 100 nM, 1 μM) and Tat (100 or 200 nM) combined caused significant losses in neurons (P<0.05). The toxicity of 1 μM morphine plus 200 nM Tat was reversed with 3 μM naloxone. Neither 1 μM morphine nor 200 nM Tat alone increased toxicity at 24 hr. These findings provide evidence that opioids and HIV-1 Tat protein are synergistically toxic via direct effects on striatal neurons, and that the interactive effects are mediated through opioid receptors. Supported by NIDA (DA 06204).

Mon49

opioids regulate oligodendrocyte survival through autocrine signaling.

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Our previous work has shown that oligodendrocytes (OLs) express μ and κ opioid receptors. In developing OLs, μ receptor activation increases OL proliferation, while the κ antagonist nor-binaltorphimine (nor-BNI) affects OL differentiation (*Glia*, 22:189, 1998). Because opioids were not present in our defined culture medium, we hypothesized that nor-BNI blocked opioids produced by the OLs themselves. To test this, proenkephalin and prodynorphin peptides were assessed in OLs using immunocyto-chemistry and/or by Western analysis. Immature OLs possessed large amounts of proenkephalin precursor and dynorphin A peptides. With OL maturation, however, there was increased processing of proenkephalin precursors into smaller peptide fragments, while dynorphin was undetectable. To assess the function of OL-derived opioids, the effect of κ agonists/antagonists on OL differentiation and death was explored. κ agonists alone had few effects. In contrast, nor-BNI significantly increased OL death, and additive OL losses were evident when nor-BNI was paired with toxic levels of glutamate, suggesting that κ receptor blockade is proapoptotic. The results suggest that OLs express proenkephalin and prodynorphin peptides in a developmentally regulated manner, and further suggest that opioids modulate OL maturation and survival through paracrine and/or autocrine mechanisms. Supported by NSF (IBN9603750) & NIH (DA-06204).

Mon50

NALOXONE'S EFFECT ON OPERANT RESPONDING TO FOOD REWARD IN THE DBA/2 STRAIN OF MICE

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Mice are powerful models to investigate the basis of food reward because there are many spontaneous obesity mutants and the murine genome is accessible to selectively targeted manipulations. Experiments in rats have shown that opioid receptor blockade reduces operant responding to food reward and has led to the suggestion that endogenous opioids influence the motivation to acquire food through modulation of brain reward pathways and not by altering the metabolic demand for nutrition. As a preface to studies of mice harboring targeted mutations in opioid peptide genes, we asked whether naloxone influences operant behavior in mice similarly to rats. We chose to examine the DBA/2J strain of mice since their analgesic response to opioids falls within the average range of several inbred strains. Twelve food-restricted male mice were shaped to lever press for food pellets and then returned to an ad lib feeding schedule. Subsequently they were randomized in a within subjects design for no injection, saline or naloxone (10 mg/kg i.p.) 20 min before each daily trial. There was a significant main effect of injection to reduce breakpoints compared to the noninjected trials on a progressive ratio of three (PR3) schedule. However, there was no significant difference in breakpoints between naloxone and saline trials. Thus, the stress of injection alone reduced the breakpoint and may have obscured an independent effect of naloxone on this measurement of motivation. Interestingly, the number and percentage of dispensed pellets eaten were dramatically reduced following naloxone compared to saline injections for either chow-based or sucrose pellets. These data suggest that opioids may influence appetite in mice but not the animals' motivation to acquire reinforcers when presented with an increasing work requirement. Supported by NIH DA05841 & DK55807

Mon51

PHARMACOLOGIC PROFILES INDICATE VAGOLYTIC OPIATE RECEPTORS IN THE S.A. NODE ARE DELTA SPECIFIC.

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Met-enkephalin-arg-phe (MEAP) is an endogenous opiate derived from the C-terminal sequence of proenkephalin. This heptapeptide is abundant in the myocardium and has significant vagolytic activity when infused systemically. The practical significance of the vagus is illustrated by the fact that patients, who regain vagal control of heart rate, have an increased chance of survival. Previously we have reported that MEAP and deltorphin (a delta opioid receptor agonist) are both vagolytic, when introduced into the S.A. Node. The maximal deltorphin and MEAP effects were both reversed by the paired infusion of the delta opiate receptor antagonist, naltrindole. The following studies were conducted to rule out participation by other opiate receptor subtypes. Microdialysis probes were placed in the sinoatrial (SA) node of mongrel dogs and perfused at five μl/minute and dose responses were conducted for both selective mu and kappa agonist and antagonist. There were no significant effects on vagal function observed when similar doses of mu (endomorphin-1, super DALDA) and kappa (U50,488, Dynorphin) receptor agonists were infused into the sinoatrial node (n = 20). We have also demonstrated that nor-binaltorphimine (kappa antagonist) and CTAP (mu antagonist) were unable to block the vagolytic effects of nodally administered MEAP (n = 10). These data suggest that the vagal effects of exogenous MEAP involve the activation of delta opiate receptors within the SA node.

Mon52

ROLE OF OPIOIDS IN HYPOTHALAMIC CONTROL OF GNRH RELEASE MECHANISM IN OVARIECTOMIZED RATS

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All the facets of activity exhibited by the cells are susceptible to pharmacological manipulations. During the present investigation, the effect of intracerebroventricular administration of mu-opioid agonist, morphine (a drug of potential abuse), and its antagonist, naloxone followed by morphine was studied on gonadotropin releasing hormone (gnrh) neurons in hypothalamic areas such as medial preoptic area (mpoa) and median eminence arcuate (me-arc) regions of brain from ebp-primed ovariectomised rats. We also assayed serum luteinizing hormone (lh) and follicle stimulating hormone (fsh) after morphine and naloxone + morphine treatments. A number of immunoreactive gnrh cells and their terminals showed a decrease in mpoa and me-arc regions respectively. The present results revealed that the morphine administration suppressed the lh levels. Naloxone given prior to morphine injection resulted in the recovery in the serum lh levels and in the number of immunoreactive gnrh neurons. Our study provides evidence for opioidergic modulation of gnrh release and results may further help to elucidate the basis of neuronal reproductive dysfunction in opiate addicts. Moreover clinical findings showing an impairment of hypothalamic-pituitary-gonadal axis following psychological or physical stimuli may be explained by the inhibitory effects of opioids on gnrh secretion.

Mon53

THE ANABOLIC-ANDROGENIC STEROID NANDROLONE AFFECTS THE DOPAMINE RECEPTORS IN THE MALE RAT BRAIN

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Anabolic-androgenic steroids (AAS) are currently used and abused not only in order to improve appearance or sports performance, but also to become intoxicated and braver. The AAS use has been highly associated with psychotropic substance use. Recent reports indicate that AAS might possess rewarding properties, but these aspects have to be further investigated.

The aim of the present study was to assess if the AAS nandrolone would affect the expression of the dopamine receptors in areas of the male rat brain implicated in reward and motoric behaviour. Autoradiography was employed in order to evaluate the effect of a two-week treatment with daily i.m. injections of nandrolone decanoate at the dose of 15 mg/kg (AAS rats) and the vehicle arachidis oleum (control rats), upon the D_1-like and the D_2-like receptor subtypes, respectively.

The findings of this study showed that the total specific radioligand binding of dopamine receptors were significantly altered in the AAS treated rats compared to the controls in the nigrostriatal and mesolimbic pathways.

Mon54

DYNORPHIN A_{1-13} AND NMDA AND THIER EFFECTS ON THE HPA AXIS IN FETAL SHEEP.

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It has been suggested that Dyn A_{1-13} stimulates the release of ACTH and cortisol in the ovine fetus via a mechanism involving NMDA receptors. Furthermore, CRH and AVP are not involved in this response. We hypothesize that Dyn A_{1-13} and NMDA act directly on the pituitary to induce an increase in ACTH. Fetal sheep underwent surgery at ~117d of gestation. Catheters were placed in the fetal jugular vein and carotid artery. Fetuses underwent either hypothalamo-pituitary disconnection (HPD, n=4), hypophysectomy (HX, n=4) or a sham surgical procedure (SHAM, n=4). Following surgery fetal sheep were given Dyn A_{1-13} (0.5 mg/kg, iv), U50,488H (1 mg/kg, iv), NMDA (4 mg/kg, iv) or saline (3 ml, iv). Blood samples were taken at regular intervals before and after drug administration. Basal ir-ACTH and ir-cortisol concentrations in SHAM fetuses were 34.9±1.0 pg/ml and 12.9±1.9 ng/ml, respectively. Dyn A_{1-13} caused an increase in fetal plasma ir-ACTH within 5 min (263±20 pg/ml) and ir-cortisol peaked within 10 min (21.3±2.7 ng/ml). In SHAM fetuses NMDA and U50,488H also caused a significant rise in ir-ACTH and ir-cortisol, whereas saline had no effect on either hormone. Basal ir-ACTH levels in HPD and HX fetuses were 38.6±12.5 pg/ml and <20 pg/ml respectively. Basal ir-cortisol levels were <2.1 ng/ml in both HPD and HX fetuses. Plasma ir-ACTH and ir-cortisol were not altered by any of the drug treatments in either HPD or HX fetuses. The results of this study indicate that Dyn A_{1-13} and NMDA act on the hypothalamus to release ACTH.

Mon55

OPIOID AGONIST STIMULATED GTPγS BINDING IN NEONATAL GUINEA PIG BRAINSTEM.

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Narcotic analgesics act upon the brainstem to induce respiratory depression in the neonate. Therefore, [^{35}S]-GTPγS binding was examined in respiratory control areas of 3, 7 and 14 day old guinea pigs. Neonates were exposed *in utero* for the last two weeks of gestation by maternal subcutaneous injections of 7.5 mg/kg of morphine or an equal volume of saline twice daily. Fresh frozen brainstem sections were incubated with 10^{-5} M DAMGO, morphine or morphine-6-glucuronide (M6G) and analyzed with a phosphor imager to calculate percent stimulation. DAMGO stimulated GTPγS binding was greater than that of either morphine or M6G in the caudal and rostral ventral respiratory groups (cVRG and rVRG), nucleus tractus solitarius (NTS) and preBötzinger Complex (pBC) of the medulla as well as in the parabrachial nucleus (PB) and Kölliker-Fuse (KF) nucleus of the pons. There were age-related changes in the rVRG and PB with stimulation of binding on day 7 being greater than on day 3. In addition, there were interactions between age and *in utero* drug exposure in the rVRG and PB where on day 7, binding was greater in morphine pretreated animals compared to saline. These data are consistent with ligand binding studies in brainstem homogenates and may be related to changes in respiratory control during development. (Supported by DA07912.)

Mon56

INTRACEREBROVENTRICULAR INJECTION OF ANTI-SENSE HuD OLIGONUCLEOTIDES IN MICE RESULTS IN SEVERE MOTOR DYSFUNCTION AND SEIZURES.

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The neuronal protein HuD is a target of the immune response observed in patients with associated paraneoplastic encephalomyelitis and sensory neuronopathy. Studies have shown that HuD plays a role in the establishment and maintenance of the neuronal phenotype. In order to examine the function of HuD *in vivo*, we injected phosphorothioated HuD antisense oligonucleotides in male CD-1mice (42ug; n≥10). Control groups consisted of saline-, mismatch- and sense-treated animals (10 animals/gp). In the antisense group, 8/10 mice developed neurological symptoms, with a peak time of 30 minutes (hyperexcitability) and progressing until 120 minutes after injection. HuD antisense-treated mice displayed symptoms which included seizures, ataxia, and paralysis. All symptoms were completely resolved within 24 hours. The mismatch, saline and sense controls failed to exhibit any of these symptoms. In prior studies using female Balb/c mice and oligonucleotides from a different manufacturer similar results were seen. Biochemical analysis of HuD expression in the animals nervous system is in progress. Our findings suggest that HuD plays a critical role in neuronal functioning and a downregulation of HuD may have significant effects toward the understanding of paraneoplastic syndromes. (Supported by NIH DA00310).

Mon57

NALOXONE FAILS TO INDUCE CONDITIONED PLACE AVERSION IN MICE LACKING THE MU-OPIOID RECEPTOR.

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There is growing evidence for tonic activity of the opioid system. Naloxone (NLX) is known to produce an aversive affective state in non-dependent subjects. NLX acts centrally to mediate aversion, and is thought to produce this effect via blockade of mu-opioid receptors (MOR). We sought to further elucidate the role of the MOR in mediating NLX aversion. Mice lacking the MOR were tested for NLX induced aversion as assayed by the conditioned place aversion (CPA) paradigm. Whereas a high dose of NLX produced a robust place aversion in wild type control mice, NLX had no aversive effect in mice lacking the MOR. We conclude that the MOR is crucial for the acquisition of NLX induced CPA. (Supported in part by NIDA Grants DA 05010 and DA09359; PDS was supported by a NIDA training grant T32DA07272 and the Hatos Center)

Mon58

MOTIVATIONAL RESPONSES INDUCED BY MORPHINE AND COCAINE IN CB1 KO MICE

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The involvement of the CB1 cannabinoid receptors in morphine and cocaine motivational responses has been investigated by using CB1 knockout mice. For this purpose, we have evaluated morphine and cocaine rewarding effects in the place conditioning paradigm and the sensitization to the locomotor responses induced by these drugs. Acute locomotor effects of morphine were preserved in CB1 knockout mice, but the sensitization to its locomotor responses was abolished in these mice. Morphine also failed to produce conditioned place preference in mice lacking the CB1 receptors. In contrast, acute locomotor effects of cocaine and chronic cocaine-induced locomotor sensitization were similar in both genotypes. Furthermore, cocaine produced similar rewarding responses in the conditioning place preference in both wild-type and knockout mice. These results demonstrate that CB1 cannabinoid receptors are essential for adaptive responses produced by chronic morphine, but not by chronic cocaine treatment.

Mon59

EFFECT OF ALUMINIUM CHLORIDE ON MICE MEMORY BEHAVIOR AND THE LONG-TERM POTENTIATION OF RATS HIPPOCAMAL SLICES .

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Abstract Objective: To observe the effect of 1%$AlCl_3$ on mice memory behavior, we injected $AlCl_3$ in mice lateral ventricle (i.c.v) in a larger dose every other day. On the basis of praxiology, we studied the influence of various concentration $AlCl_3$ to the long-term potentiation of rats hippocamal slices from a cytologic pattern by electrophysiological method. **Method:** This paper described a method that model of learning and memory disturbance was made by injecting 1%$AlCl_3$ (i.c.v) . The mice of model group was injected 1%$AlCl_3$ solution (2uL/mouse) in one lateral ventricle on d1 while d3 in other side. The control was injected Saline (2uL/mouse) by the same way. A passive avoidance response test and a locomotor activity counting can be carried out on d5. We infused different concentration $AlCl_3$ (10^{-8} mol/L ,10^{-6}mol/L and 10^{-4} mol/L) to rats hippocampal slices respectively at 1ml/min rate. An alteration of population spike amplitude (PSA) was observed. **Result:** In the step down and step through test, the number of errors obviously was increased and the latency obviously was shortened in model group and had significant difference ($p<0.05$) compared with that of control group, but the normal group had no significant difference ($p>0.05$) compared with that of control. In the locomotor activity counting, number of locomotor activity of all groups had no significant difference ($p>0.05$) compared with that of control. In the experiment of rats hippocampal slices in vitro, the RPSA was reduced obviously in treatment groups with different concentration $AlCl_3$ compared with baseline($p<0.05$), and had a relation of dose-effect . **Conclusion:** The result showed that $AlCl_3$ can cause an evident memory disturbance of passive avoidance response to mice following injection i.c.v every other day and inhibit the LTP of rats hippocampal slices.

Wed 01

RECEPTOR DESENSITIZATION AS A POSSIBLE MECHANISM OF TOLERANCE TO MU OPIOIDS Abdel-Azim Assi, Wenzhen Jin and Charles Chavkin. Department of Pharmacology, University of Washington School of Medicine Box 357280, Seattle, WA 98195-7280

The mechanisms underlying tolerance caused by prolonged opioid receptor activation were investigated by studying mu receptor desensitization in the dentate gyrus of the mouse hippocampus. The effects of opioid agonists with different efficacies (morphine and fentanyl) on the amplitude of granule cell population response evoked by stimulating the perforate pathway were compared and evaluated. Mice of 21-28 days old were used. Dose-response analysis of opioid effects showed that the peak response to fentanyl (300 nM) was larger than that for a maximally effective morphine (2 μM) consistent with prior reports showing that morphine has a lower efficacy than fentanyl. On the first evoked synaptic response, the initial fentanyl (300 nM) peak effect was increased by 158 ± 37% of the base line (n=7) and that to morphine (2 μM) was increased by 138 ±11 % of the base line (n=7). On the second synaptic response, the initial fentanyl (300 nM) peak effect was 221±36 % of the base line (n=7) and that of morphine (2 μM) was 197 ±24 % of the base line (n=7). Both responses were gradually reduced with continuing perfusion of fentanyl, and only 33.5-40 % of the initial increase remained after 60 min of continuous treatment. The effect of morphine on the second response remained unchanged and no desensitization was detected after 60 min of infusion. The effect of morphine on the first response, however, was desensitized after 45 min of treatment. Such desensitization was slow and less sharp than that of fentanyl which started 15-20 min after infusion. Decreasing fentanyl concentration to 100 nM reduced desensitization to its effects by about 40%. Longer treatment of the hippocampus with morphine did, however, cause desensitization in both the first and second evoked responses. Naloxone (1-2 μM) antagonized the effects of both fentanyl and morphine and moved the response to below the base line. The NMDA antagonist, aminophosphovaleric acid (APV, 50 μM), partially and significantly reduced the desensitization to all fentanyl effects with no marked effect on the second evoked response of morphine (within the test time). APV, however, significantly attenuated the desensitization developed to morphine in the first evoked response. We conclude that desensitization is one of the mechanisms of tolerance to mu opioids, and it develops rapidly to opioids with high efficacy. The results also demonstrate that NMDA-receptors play role in the development of opioid desensitization and tolerance in the in-vitro brain slice preparation.

Wed02

THE EFFECT OF OPIOIDS ON Ca^{2+}/cAMP RESPONSIVE ELEMENT BINDING PROTEIN REGULATION

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Ca^{2+}/cAMP response element binding protein (CREB) is an important factor linking the opioid-regulated secondary messenger systems to alterations in gene expression. The effect of opioids (morphine, DAMGO and endomorphin-1) on the CREB level, its phosphorylation and binding to its corresponding response element was investigated in Neuro2a MOR1 cells. Acute administration of opioids increased CREB phosphorylation and binding to consensus CRE and AP-1 elements without affecting total CREB protein level. Acutely, opioids stimulated also CRE and AP-1-directed transcription of luciferase reporter gene. Morphine and endomorphin-1 induced CRE and AP-1 DNA binding activity more potently than DAMGO. Prolonged opioid treatment normalized back to basal the levels of CRE and AP-1 DNA binding activity and slightly decreased the levels of phosphorylated CREB. Withdrawal from the drug elicited an increase in phosphorylated CREB levels and induced CRE and AP-1 DNA binding activity. As total CREB protein level was not affected by opioids the induction of CRE DNA binding activity suggests the involvement of other factors. Our findings provide evidence that regulation of gene expression may contribute to development of tolerance and addiction. Our results also highlight that not only CREB but also other putative factors may be crucial for transcriptional adaptation to opioids.

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Wed03

RESISTANCE TO MORPHINE TOLERANCE IN MICE LACKING βARRESTIN-2

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Previously we demonstrated that functional deletion of the βarrestin-2 gene in mice results in remarkable potentiation and prolongation of the analgesic effects of morphine suggesting that μOR desensitization may be impaired in these mice *(Bohn et al., Science, 286, 1999)*. A large body of evidence exists to indicate that βarrestins are directly involved in desensitization of GPCRs in vitro. Here we have examined the contribution of βarrestin-2 to desensitization of the μOR and the development of tolerance in this genetic mouse model. In βarrestin-2 KO (βarr2-KO) mice, as opposed to their wild-type (WT) littermates, chronic administration of morphine (5 days) failed to produce tolerance to morphine-induced antinociception. In WT littermates, chronic exposure to morphine blunted G-protein coupling of the μOR (^{35}SGTPγS binding) in brainstem membranes, while normal μOR coupling was preserved in βarr2-KO mice. These observations suggest that βarrestin-2 is physiologically important in regulating the function of the μOR. Moreover, they suggest that disruption of μOR desensitization prevents the development of opiate tolerance. *Supported in part by NIH grants DA006023 (L.M.B.), HL16037 (R.J.L.), and NS19576 (M.G.C.).*

Wed04

Preliminary Studies of Rebirthipe on Naloxone-Precipitated Withdrawal Symptoms in Morphine-dependent Mice

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Owing to the high potential of opioid tolerance and dependence, it is an important task to develop new analgesic drugs with lower potential for abuse and less side effects than the commonly available opioids. From our preliminary test, we found that several Chinese herbs might give a new solution to this. In this study, we found a formula of Chinese herbs, Rebirthipe, had sedative and anticonvulsive effects and decreased the spontaneous locomotor activity mice. Rebirthipe also showed antinociceptive effects on both hot-plate test and writhing response. Furthermore, Rebirthipe attenuated the abstinence behavior of morphine evoked by naloxone in mice. From the above results, Rebirthipe might have a value on the detoxification of opioid tolerance.

Wed05

POTENTIAL ROLE OF RGS4 IN DESENSITIZATION OF THE MU OPIOID RECEPTOR ON SH-SY5Y CELLS

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DAMGO caused a concentration-dependent inhibition of forskolin-stimulated adenylyl cyclase activity in homogenates of SH-SY5Y cells (EC50 = 30 nM, Bmax = 65% inhibition). Preincubation of cell homogenates with 3 μM recombinant RGS4 increased basal adenylyl cyclase activity by ~80% and enhanced forskolin-stimulated adenylyl cyclase activity by ~20%. Also, RGS4 modestly diminished maximal DAMGO inhibition of cyclase activity from 65% to 50%, but did not alter the EC50 of DAMGO. After incubating SH-SY5Y cells with DAMGO for 24h, DAMGO inhibited adenylyl cyclase activity by 50%. However, when the homogenate from DAMGO-pretreated cells was incubated 10 min on ice with 3 μM RGS4, DAMGO maximally inhibited adenylyl cyclase activity by only 20%. RGS4 did not significantly alter the EC50 value of DAMGO. Thus, pretreatment of SH-SY5Y cells with DAMGO enhanced the ability of RGS4 to suppress the efficacy of mu opioid receptor-mediated inhibition of adenylyl cyclase activity.

Wed06

REQUIRED TREATMENT FOR SUBSTANCE ABUSERS

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Studies show typically less than 10 percent of alcohol and other drug abusers voluntarily enter treatment and, once in treatment, drop out rates are high. One way to get clients into treatment is the use of legally encouraged or required treatment. The primary rationale for required treatment is that some substance abusers require external pressure to enter, participate, and remain in treatment. Discussed is the history of required treatment for alcohol and other substance abuse, the primary issues surrounding its use, the use of required treatment in the criminal justice system, the primary treatment models for alcohol and other substance abuse, ways to increase internal and external motivation for treatment, and therapist and other treatment personnel responsibilities in using required treatment.

Wed07

SEROTONIN TRANSPORTER BINDING SITES IN FETAL RHESUS MONKEY BRAIN: EFFECT OF GESTATIONAL COCAINE EXPOSURE

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Our previous studies have shown that maternal cocaine use leads to an up-regulation of the dopamine transporter in the midbrain of the day 70 fetal rhesus monkey (Fang and Rønnekleiv, J Neurosci. 19:8966-8978, 1999). The present study evaluated the distribution of serotonin transporter (SERT) binding sites and the effect of prenatal cocaine exposure on SERT binding densities in day 70 fetal monkey brain by quantitative autoradiography using [^{3}H]paroxetine, a specific SERT ligand, (4 nM, 19.7 Ci/mmol). Pregnant monkeys were treated with (-)-cocaine (3 mg/kg, i.m., 4 times/day) or saline from day 22 of pregnancy until day 70 at which time the fetus was delivered by cesarean section, and the fetal brain prepared for transporter binding autoradiography. High levels of [^{3}H]paroxetine binding (5,000~10,000 nCi/g) were found in the midbrain including the dorsal raphe > linear raphe ≥ ventral tegmental area. Moderate levels (2,000~3,000 nCi/g) were found in the lateral hypothalamus ≥ lateral preoptic area > interpeduncular nucleus ≥ olfactory tubercle > globus pallidus ≥ substantia nigra. Low levels (< 2,000 nCi/g) were found in the amygdala > superior colliculus > accumbens > medial preoptic area ≥ septum ≥ prefrontal cortex ≥ anterior cingulate. There was no significant difference in the brain regions examined between saline and cocaine-treated fetuses (n=3-5). The data suggests that the SERT is highly developed in the day 70 fetal monkey brain and cocaine appears not to affect SERT binding densities at this stage in gestation. Supported by NIH Grant DA07165.

Wed08

EXPLORATION OF MECHANISM ON LOW PHYSICAL-DEPENDENCE INDUCED BY DIHYDROETORPHINE.

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Dihydroetorphine (DHE) is a opioid receptor agonist. It is several thousand times more potent than morphine as an analgesic, with a low physiological dependence. Many papers have demonstrated that locus coeruleus (LC) may play an important role in physical-dependence on opioids. In this study, the effects characteristics of DHE were compared with morphine on monoamine transmitter release and neuron firing in different brain regions by microdialysis and means of unit recording. The results showed that the maximal increases in LC firing rate and norepinephrine (NE) release were observed by naloxone precipitated withdrawal in chronic morphine treated rats, but no effect in chronic DHE treated rats. Using radioligand binding assay, DHE showed as a selective ligand for the mu-opioid receptor. However, the relative affinity ratio of morphine and heroin to binding site of [^{3}H]-DHE was significantly different from that of [^{3}H]-DAGO (Ki value was low about 100-1000 times). The amount of cAMP generated was measured by competitive protein binding assay in NG108-15 cells. The naloxone could produced marked rebound response of cAMP on chronic treatment with morphine for 24 h in the cells, but no change in the levels of cAMP was found following DHE treated the cells for 24 h. The results suggested that the effects of DHE were different from morphine on LC and subtype of opioid receptor. It may be one of the reasons that physiological dependence induced by DHE was lower than that of induced by morphine.

Wed9

NALTREXONE DECREASES GAMBLING BEHAVIOR.

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Despite many adverse consequences related to compulsive gambling, the number of addictive gamblers is at an all-time high; estimates suggest more than 5 million "problem gamblers" in the U.S. alone. The increasing incidence of gambling and the commensurate rise in detrimental outcomes emphasizes the need for understanding the basis for pathological gambling. We tested the hypothesis that gambling behavior may involve opioid release in a double-blind, placebo-controlled study. Thirty-seven "regular gamblers" (both sexes) were recruited at a local video gambling parlor. They were paid $50 and given either naltrexone (50 mg) or placebo before filling out questionnaires for 30 min and then allowing the experimenter to record their gambling behavior. Measures of gambling assessed were the time spent playing, amount of money spent, size of bets and amount of money cashed out. Naltrexone reduced the amount of money bet by about 40%. Gender effects were evident in bet size and total cash input, with women spending significantly more than men. These data suggest that endorphins contribute to the addictive process underlying compulsive gambling and that pharmacotherapies involving opioid antagonists may hold promise for treating this illness.

Wed10

COUPLING OF μ-OPIOID RECEPTOR TO GIRK IN EGFP-POMC NEURONS IN THE MOUSE

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The μ-opioid receptor is an autoreceptor in hypothalamic POMC neurons that becomes uncoupled and downregulated in the female guinea pig with chronic morphine treatment. In order to directly visualize POMC neurons, we produced a transgenic mouse with an Enhanced Green Fluorescent Protein (EGFP) inserted into the 5' noncoding region of POMC exon 2. The transgenic mice (4 generations on the inbred C57BL/6J background) exhibited highly specific expression of EGFP in >99% of β-endorphin immunoreactive POMC neurons throughout the rostral-caudal extent of the hypothalamic arcuate nucleus. Using whole-cell patch recording and in the presence of TTX, DAMGO (1 μM) produced a maximum outward current of 12±4.9 pA in all EGFP-POMC neurons tested (n=25). I_{DAMGO} reversed near E_{K+} (84±4.2 mV) and showed an increase in slope conductance below E_{K+} characteristic of the inward rectifier. Therefore, the μ-opioid receptor is coupled to GIRK in mouse arcuate POMC neurons. Future experiments will determine the effects of chronic morphine treatment on this coupling. (Supported by: P01DK55819, F31HG00201, FIRCA R03TW001233, DA05158 and NS 38809)

Wed11

CURRENTS ASSOCIATED WITH THE DOPAMINE TRANSPORTER IN CULTURED DOPAMINE NEURONS.

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Uptake by the neurotransmitter transporters is the principal mechanism by which biogenic amine neurotransmitters are cleared from the synaptic cleft so that their signal may be terminated. In addition to this action, more recently it has been determined that the expressed cloned transporter proteins also elicit measurable ion channel-like currents. Whole-cell patch-clamp recordings from dopamine neurons in culture were used to determine if currents associated with the dopamine transporter could be measured. Superfusion of dopamine (10 μM) and amphetamine (10 μM) consistently elicited small inward currents at -60 mV that were blocked by cocaine (10 μM) and the selective DAT inhibitor, GBR 12909 (1 μM). Cocaine alone elicited a small outward current at -60 mV and large inward currents at positive potentials suggesting blockade of a leak conductance through the transporter. These results suggest that currents associated with the dopamine transporter may contribute to the electrical properties of DA neurons. Supported by DA07595 and HHMI.

Wed12

CHANGES IN AMPA RECEPTORS FOLLOWING MORPHINE WITHDRAWAL IN THE RAT BRAIN

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To examine AMPA-sensitive glutamatergic involvement in expression of the withdrawal syndrome from opioids, an autoradiographic study of [^{3}H]AMPA receptor binding and an assessment of *in situ* hybridization of AMPA sensitive glutamate receptor A (GluR-A) subunits in the rat brain were each performed 7 h after withdrawal from morphine infusion. Animals were rendered dependent by intracerebroventricular (i.c.v.) infusion of morphine (26 nmol/μl/h) via osmotic minipumps for 3 days. Brain sections of 14 μm thickness were incubated with 15 nM [^{3}H]AMPA for quantitation of binding to the AMPA receptor. The probe for *in situ* hybridization was labeled at its 3' end using terminal deoxynucleotidyl transferase and [^{35}S]dATP. The extent of [^{3}H]AMPA binding was increased significantly in the cortex areas (18-21%), caudate-putamen (20%), and hippocampus (7-9%) of rats following withdrawal from morphine. The highest levels of mRNA for GluR-A, flop and flip subunits, were found in the dentate gyrus and in the CA3 region of the hippocampus, respectively. The levels of mRNA for the flop form of GluR-A were decreased in the CA3 of hippocampus (8%) of the rat brain. The levels of mRNA for the flip form of GluR-A were increased in the parietal cortex (7%) and the entorhinal cortex (8%). Changes in the binding of [^{3}H]AMPA and mRNAs to its receptor may contribute to withdrawal syndrome from morphine.

Wed13

MU OPIOID DESENSITIZATION SHARES COMMON MECHANISM WITH LTD IN THE HIPPOCAMPAL DENTATE GYRUS.

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Molecular mechanisms underlying mu opioid desensitization were investigated in the dentate gyrus of the mouse hippocampus. Application of the strong mu opioid agonist fentanyl (FENT, 300 nM), increased the amplitude of the granule cell population response evoked by perforant path stimulation to 221±35% of baseline (n=7). Continuous perfusion of FENT for 60 min gradually reduced the increased response by 68.0±9.0% of the initial peak response. We found that 50 µM APV, an NMDA receptor antagonist, and 50 µM MCPG, a mGluR antagonist significantly attenuated the FENT desensitization, suggesting that activation of these glutamate receptor subtypes was critical for the mu opioid desensitization observed. Because activation of NMDA receptor and mGluR are also known to be important for synaptic plasticity, we next examined the relationship between opioid desensitization and synaptic plasticity. LTD was induced by low frequency stimulation (1Hz, 15 min) of the perforant path. LTD was NMDA dependent in these studies. We found that FENT-induced desensitization prevented the induction of LTD. Our results suggest that mu opioid desensitization in the dentate gyrus share a common mechanism with LTD. Thus, synaptic plasticity may play an important role in opioid tolerance and dependence. Supported by DA04123 and DA00266.

Wed14

EFFECT OF ZINC ON MORPHINE A EFFECT OF ZINC ON MORPHINE ANALGESIA, TOLERANCE AND DEPENDENCE.

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N-methyl-D-aspartate (NMDA) receptor antagonists inhibit the development of tolerance and dependence on narcotic analgesics. Zinc is a noncompetitive antagonist of the NMDA receptor but also inhibits opioid binding. Because high concentrations of vesicular zinc are localized in the dorsal spinal cord, we studied the effect of zinc on morphine analgesia, the development of acute morphine tolerance and chronic dependence. Using a dose of zinc that inhibits the behavioral response to i.t. administered NMDA, zinc was administered i.t. 1 hr before 10 mg/kg of morphine i.p. and tested 30 min later using the tail flick assay. Although zinc was antinociceptive in the writhing assay, zinc inhibited morphine antinociception in the tail flick assay in a dose-related fashion. Zinc also inhibited the development of tolerance to morphine when tested 5 hr after an injection of 100 mg/kg of morphine i.p. Injected i.t. twice daily, zinc had no effect on the development of chronic dependence induced by a 75 mg morphine pellet. To determine the influence of endogenously occurring zinc, a cell impermeant chelator of divalent cations was injected i.t. Chelation of zinc with Na^{+} Ca^{++} EDTA did not have any effect on morphine analgesia or acute tolerance. These data suggest that increasing the concentration of zinc above that normally found in the CNS, attenuates opioid activity. [NIDA07234 (KJK); NS39740 from NINDS/NIAMS (AAL)]

Wed15

RB101(S) DOES NOT INDUCE ANTINOCICEPTIVE TOLERANCE, OR CROSS-TOLERANCE WITH MORPHINE.

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In behavioral tests, RB101 a complete inhibitor of enkephalin-degrading enzymes have strong antinociceptive effects without producing tolerance, or cross-tolerance with morphine. This was reexamined with the enantiomer RB101(S), using c-Fos studies at the spinal cord level in a model of inflammatory nociception (intraplantar injection of carrageenin, 6 mg/150 µl of saline, in awake rats). Acute drugs were injected 10 min before carrageenin, and rats were perfused 90 min later. C-Fos-protein immunoreactivity (FIR) was evaluated in the lumbar spinal cord. FIR neurons were preferentially located in the superficial (I-II) and deep (V-VI) laminae of segments L4-L5 (areas containing numerous neurons responding exclusively, or not, to nociceptive stimuli). In the first experiment (n=23 rats), acute RB101(S) (30 mg/kg, i.v.) or vehicle was injected in naive or morphine tolerant (100 mg/kg/day for 3 days, s.c.) rats. RB101(S) reduced the total number of FIR neurons with similar magnitude in naive (40% of reduction, $p<0.01$) and in morphine-tolerant rats (35% of reduction, $p<0.01$). In the second experiment (n=40 rats), RB101(S) (30 mg/kg, i.v.), morphine (3 mg/kg, i.v.), or respective vehicles were injected in rats chronically treated with RB101(S) (160 mg/kg/day for 4 days, s.c.). In chronically treated RB101(S) rats, both acute RB101(S) and morphine, reduced the total number of FIR neurons (26% and 35% of reduction, $p<0.0001$, respectively). These data provide further evidence that different cellular mechanisms occurred after chronic stimulation of opioid receptors by morphine or endogenous enkephalins. Supported by ARC #9605, and MILDT #98D11.

Wed16

GRK2 IS THE UNIQUE GRK INVOLVED IN THE DESENSITIZATION OF HUMAN DELTA OPIOID RECEPTOR.

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Previously, we demonstrated that the phosphorylation of the human delta opioid receptor, endogenously expressed in the neuroblastoma SK-N-BE, was directly correlated to its desensitization observed on the inhibition of adenylyl cyclase. This phosphorylation is mediated by one or more member(s) of the GRK family (Hasbi et al., 1998). Determination of the GRK expression in the SK-N-BE cells was conducted by immunoblotting and revealed that only GRK2 is expressed. To determine more precisely the involvement of this kinase in the phosphorylation and desensitization, SK-N-BE were transfected with bovine GRK2 or with the dominant negative mutant K220R. Fonctional experiments conducted on the adenylyl cyclase, show that overexpression of this kinase strongly reduced the inhibition of adenyl cyclase by the receptor, while K220R expression inhibits its desensitization. Activation of GRK2 by delta opioid receptors was also confirmed by biochemicals studies, showing a translocation of the kinase from cytosol to plasma membrane with a time course correlating with the desensitization. To our knowledge, this is the first demonstration for role of the GRK2 in desensitization of the human delta opioid receptor in a model which endogenously expresses this receptor.

Hasbi A., Polastron J., Allouche S., Stanasila L., Massotte D. et Jauzac Ph. (1998) Desensitization of the delta-opioid receptor correlates with its phosphorylation in SK-N-BE cells : involvement of a G protein-coupled receptor kinase. J. Neurochem., 70, 2129-2138.

Wed17

PKC-MEDIATED INHIBITION OF MU-OPIOID RECEPTOR INTERNALIZATION AND MORPHINE ACUTE TOLERANCE

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As reported elsewhere, the incubation of mu-opioid receptor (MOR)-expressing CHO cells with 1 microM DAMGO, a peptide MOR agonist, caused a marked internalization of the receptor 30 min after the agonist challenge, detected by immunocytochemistry. However, the incubation with 10 microM morphine did not affect the receptor dynamics. When calphostin C, a protein kinase C inhibitor was treated with morphine, MOR was internalized at as early as 10 min after the agonist challenge. Both internalization by DAMGO alone and morphine plus calphostin C were abolished by the pretreatment with adenovirus expressing K44A dynamin negative mutant. On the other hand, in the peripheral nociception test in mice, nociceptive flexor responses by intraplantar injection of bradykinin (BK) were blocked by similar application with morphine or DAMGO. When morphine was given again at 4-hr later after the initial challenge with morphine, morphine-induced analgesia was markedly reduced (acute tolerance). The acute tolerance was inhibited by calphostin C-pretreatment. Acute tolerance was not observed with the similar treatment with DAMGO, however, it was elicited by the K44A adenovirus pretreatment and then rescued by calphostin C. These results suggest that the acute tolerance is mediated by PKC-dependent inhibition of receptor internalization.

Wed18

PHOSPHORYLATION SITES AND REGULATION OF THE DELTA OPIOID RECEPTOR .

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Like other G-protein coupled receptors, delta opioid receptor (DOR) is phosphorylated upon exposure to agonits. In the present study, we investigated the DPDPE-induced phosphorylation of DOR. We constructed many mutants of the potential phosphorylation sites (Ser/Thr) at the C-terminal of the receptor, individually or in combination. The constructs were transiently or stably transfected in HEK293 cells. The affinities for diprenorphine and DPDPE of all the mutants were similar to that of the wild-type receptor. The exact sites involved in the DPDPE-induced phosphorylation of the receptor were determined and we found that the observed phosphorylation is sequential. We investigated also the molecular mechanisms underlying DOR desensitization and internalization, and showed that phosphorylation of the receptor contribute to the regulation of these events. Supported by NIDA-NIH grants.

Wed19

IMBALANCE IN ENDOGENOUS CCK/OPIOID SYSTEMS AND VULNERABILITY TO DRUG-TAKING

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Drug-preferring Lewis rats and drug non-preferring Fischer rats can serve to study genetically-determined differences in behaviour to drugs of abuse. To study possible differences in levels of endogenous opioids and CCK systems between Lewis and Fischer rats we studied the analgesic effects induced by endogenous and exogenous opioids, associated or not with CCK_B antagonists in both strains. Moreover, we directly determined the levels of enkephalins and CCK in the nucleus accumbens by microdialysis in freely moving rats. The results seem to demonstrate an imbalance between the endogenous CCK and opioid systems in Fischer rats as compared to Lewis rats, which could be at the basis of individual differences in vulnerability to drug-taking. Further experiments are in progress to firmly demonstrate this hypothesis.

Wed20

DISSOCIABLE EFFECTS OF OPIATE WITHDRAWAL ON DRUG-SEEKING (DS) AND DRUG-TAKING (DT)

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The aversive symptoms of opiate withdrawal are alleviated by the drug itself, suggesting that relapse to drug use is motivated by the negative effects of drug abstinence. It is therefore surprising that studies examining the role of withdrawal in relapse to drug use have produced inconsistent results. This may be due to the fact that most animal models do not dissociate DS and DT. We used a paradigm that independently measures DS and DT to explore how these two behaviours are affected by level of withdrawal. Long-Evans rats were trained to respond for IV heroin (0.12mg/infusion) under a chain (FR 1; VI 120s) schedule in which responding in the first seeking link gave access to the opportunity to perform a different taking response in the second link. The second link terminated with a drug infusion. DS was decreased at 6 and 12 h after the last infusion but increased at 24h. Conversely, DT was increased only at the 6 h test. Sucrose consumption was decreased at each test, compared to a group of drug-naïve animals. Somatic withdrawal scores also differed significantly from controls at each test. Results suggest that opiate withdrawal has different effects on DS and DT and that the two behaviours may be governed by different motivational states. Additional tests will be conducted at 3, 6, 12, and 25 days after the last self-administration session.

Wed21

ERK INHIBITION REDUCES OPIOID TOLERANCE IN RATS

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Background: Extracellular signal-related kinase (ERK) is a member of the mitogen- activated protein kinase (MAPK) superfamily, which are involved in regulating many cellular functions. We and others have shown that ERK is strongly activated by μ opioids. To date, this is the only signalling system activated by acute opioid administration. The time course of ERK activation parallels the time course of morphine's clinical effects, suggesting a possible mechanistic role. ERK activation by opioids exhibit both acute and chronic desensitization, suggesting a potential role for ERK in opioid tolerance and dependence. The present study determined whether inhibition of ERK by UO126 would affect opioid tolerance development. Methodology: 21 male Sprague- Dawley rats had intrathecal catheters implanted under halothane anesthesia and recovered for a week. Subsequently, either a 75 mg morphine or placebo pellet were implanted and a primed osmotic minipump infused either 0.1M UO126, DMSO vehicle or saline at 1μl/hr. was connected to the catheter. Tolerance development was assessed using tail flick latency. Three days after the initial pelleting, 3 additional pellets of the same type were implanted. On day 7, withdrawal was precipitated using naloxone and withdrawal symptoms observed. Results were then analyzed by repeated measures ANOVA and the Tukey-Kramer post-hoc test with $p<0.05$ considered significant. Results: UO126 alone did not possess analgesic properties and did not augment morphine analgesia. After 48 hours, there was a trend toward inhibition of tolerance by UO126; after the second pelleting, this trend reached statistical significance. However, the DMSO vehicle also mildly inhibited tolerance development, but did not posses analgesic properties. Intrathecally administered UO126 had no effect on withdrawal symptoms since many of these symptoms are thought to be mediated supraspinally. Conclusions: These results demonstrate the ERK inhibition reduces the development of opioid tolerance in rats. The confounding effect of DMSO on tolerance inhibition will be avoided by the use of novel solubilizing agents. Studies to further define the role of ERK in opioid tolerance and physical dependence are ongoing.

Wed22

MELANOCORTIN RECEPTOR MEDIATED EFFECTS IN THE ALCOHOL PREFERRING AA RATS

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The melanocortin (MC), opioid and nociceptin peptides and their receptors have been implicated in a number of functions in the CNS, including drug dependence mechanisms. The purpose of this study was to assess whether the synthetic MC4-receptor antagonist HSO14 and the synthetic unselective agonist MTII could affect the alcohol and water intake in the alcohol-preferring AA rats. We were also interested in how these MC substances could affect the opioid and nociceptin peptide levels in the alcohol drinking AA rats. 25 AA rats were allowed to self-administer alcohol (10% v/v) for one month, using a free choice between two bottles of alcohol and water. One group (8 rats) received only water. After this period the rats were administrated i.c.v. injection of either 1 nmol/rat of MTII or HSO14. Two groups of rats received only CSF. MTII lowered the alcohol intake, and induced an increase in water intake. After the HSO14 injection the rats drank less alcohol, but the effect was delayed and not as pronounced as for MTII. An initial increase was followed by a delayed decrease in water intake. The opioid and nociceptin peptides were analysed using specific radioimmunoassays. MTII and HSO14 affected the peptide levels in several brain regions in the alcohol drinking AA rats, which are believed to be involved in drug dependence mechanisms. In conclusion, the results in this study suggest involvement of the MC system in ethanol reward mechanisms.

Wed23

PRIOR COCAINE EXPOSURE IN THE FETAL RHESUS MONKEY INCREASES STRIATAL AREA CFOS EXPRESSION

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These studies were aimed at elucidating changes in dopamine neurons and dopamine target neurons in the rostral forebrain of fetuses exposed to cocaine between days 20 and 90 of gestation. To test for neuronal responsiveness, we used induction of the transcription factor cFos in response to a cocaine challenge at day 130 in saline- and cocaine-pretreated fetuses. Tissue blocks from the striatum/nucleus accumbens (nACB)/olfactory tubercle(OT) area of day 130 fetuses were fixed in 4 % paraformaldehyde, sectioned on a cryostat and reacted for cFos, tyrosine hydroxylase (TH) or the combined procedure using immunocytochemistry (ICC). Double ICC for TH and cFos in the striatum/nACB revealed a patchy distribution of TH fibers and the cFos expression was most often associated with dense TH patches. In the OT area TH positive cells were observed in all animals examined (n=20). A cocaine challenge induced cFos positive nuclei in the OT, many of which were expressed in TH neurons. Preliminary analysis suggest that the cocaine-induced cFos expression is more extensive in the cocaine-pretreated group (CC; n=5) in comparison to the saline-pretreated group (SC; n=5). These data suggest that fetal brains exposed to and withdrawn from cocaine, exhibit altered responsiveness to aminergic stimuli. Supported by NIH Grant DA07165.

Wed24

MODULATION OF MORPHINE-INDUCED SENSITIZATION BY δ-OPIOID RECEPTOR SYSTEMS

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An involvement of δ-opioid receptors (DOR) in opiate tolerance and dependence has been suggested. The present studies used a conditioned place preference paradigm in rats to examine the role of DOR in mediating sensitization to the conditioned reinforcing effects of morphine. Rats received home cage injections of morphine (0; 5.0 mg/kg; days 1-5). Fifteen min prior to injections they received graded doses of naloxone (0.1-1.0 mg/kg) or the DOR antagonists, naltrindole (0.03-0.3 mg/kg), or naltriben (0.03-0.3 mg/kg). The place conditioning produced by graded doses of morphine was assessed three days following treatment cessation. Does of morphine that were ineffective in producing conditioned rewarding effects in control animals produced robust conditioning in animals with a prior history of morphine administration indicating the development of sensitization. In animals that had received the morphine treatment regimen in combination with naltrindole, naltriben or high but not low doses of naloxone, the development of sensitization was prevented. In contrast to their effects on sensitization, neither DOR antagonist attenuated the conditioned rewarding effects produced by morphine in previously drug naïve animals. These data add to a growing body of evidence indicating an important role of DOR in mediating behavioral adaptations that occur as a consequence of repeated opiate use.

Wed25

CHANGES IN THE INTRACELLULAR DISTRIBUTION OF BRAIN MU OPIOID RECEPTORS AND G-PROTEINS ASSOCIATED WITH MORPHINE TOLERANCE

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Rats were rendered tolerant by *sc* injections of increasing doses of morphine from 10-60 mg/kg for 3, 5 or 10 days. Binding parameters of the mu opioid receptors in subcellular fractions were measured with [^{3}H]DAMGO. Up-regulation of synaptic plasma membrane (SPM) binding was detected after a 10-day drug administration. The number of mu binding sites in a light vesicle or microsomal fraction (MI) was elevated by 68% and 30% after 5 and10 days of morphine exposure, respectively. The up-regulated mu-sites in MI displayed enhanced coupling to G-proteins compared to those detected in saline-treated controls. Pertussis toxin catalyzed ADP-ribosylation and Western-blotting with specific antisera were used to quantitate chronic morphine-induced changes in levels of α subunits of G_s, G_{i1}, G_{i2}, G_o proteins in SPM and MI fractions. Our results show that distinct regulation of G-protein levels, particularly of those localized intracellularly, accompany the development of morphine tolerance. The possible mechanisms of the observed time-dependent up-regulation of the mu opioid recceptor is discussed. US-Hungarian Fund JFNo-564 and OTKA T-33062 research grant.

Wed26

TWO MATURE FORMS OF HUMAN NOCISTATIN IN BRAIN AND CEREBROSPINAL FLUID

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In order to develop nocistatin to be of clinical use as a therapeutic agent or a diagnostic tool, it is important to identify the actual nocistatin structure present in human brain and cerebrospinal fluid (CSF). we have developed a unique identification method using HPLC systems combined with RIA specific to nocistatin and identified two forms of human nocistatin in brain and CSF extracts. One form was confirmed to be identical with the tridecapeptide estimated from the human prepronociceptin gene, whilst the other structure was unexpectedly found to be the c-terminal heptadecapeptide of human nocistatin.

Extracts obtained from human brain tissues were subjected to the two HPLC systems with ODS and Phenyl columns. Nocistatin-like immunoreactivity (NST-IR) and nociceptin-like immunoreactivity (NCP-IR) were determined by RIAs. The two HPLC chromatograms showed three NST-IR peaks (1, 2 and 3), one of which, NST-IR(3), coincided with the putative 30-residue nocistatin. Two NCP-IR peaks were detected: one coincided with nociceptin and the other overlapped with NST-IR(2), suggesting NST-IR(2) should be a precursor protein common to nociceptin and nocistatin. The retention times of NS-IR(1) in the two HPLC systems were same as those of the synthetic C-terminal heptadecapeptide of nocistatin. CSF obtained from various pain state patients also showed the same NST-IR peaks.

Wed27

THE ROLE OF GLUTAMATE RELEASE AND NITRIC OXIDE ON THE MECHANISM OF U-50,488 TO PREVENT MORPHINE TOLERANCE

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Male Hartley G.P. (250~350 g) were s.c. injected with saline or morphine (15 mg/kg) or morphine + U-50,488 (0.003 mg/kg) twice daily for 7 days. Antinociceptive effect was assessed by hot–plate test. The animals were sacrificed by decapitation, and the spinal cords were removed and the lumbar part was cut to 450 μm sections and every 10 pieces were put into a culture dish for totally 3 dishes per animal. The sections were incubated with [^{3}H]glutamate for 20 min. After wash to remove free [^{3}H]glutamate, the slices were incubated with either aCSF or morphine (100 υM) or morphine + MK-801 (100 μM) for 10 min. The basal release of glutamate, [^{3}H]glutamate level and NO level in the medium were determined. NMDA-displaced [^{3}H]glutamate binding of spinal cord was also determined.

Our results indicated the increase of glutamate release, activation of NMDA receptors and increase of NO after chronic morphine treatment. On the other hand, the effect of U-50,488 to prevent morphine tolerance may be due to its effect on inhibiting glutamate release which stimulated by chronic morphine. Furthermore, U-50,488 also inhibits the overproduction of NO by chronic morphine and this may also contribute to its effect on preventing morphine tolerance.

Wed28

NMDA RECEPTOR BLOCKADE MIMICS TOLERANCE AND SENSITIZATION TO THE LOCOMOTOR EFFECTS OF MORPHINE

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There is growing evidence that N-methyl-D-aspartate (NMDA) receptors may be involved in tolerance and sensitization to the locomotor effects of opiates. The present studies represent a further exploration of this relationship. Adult male Sprague-Dawley rats received acute or chronic administration of morphine (MOR, 10 mg/kg sc), in the presence or absence of the NMDA receptor antagonist MK-801 (0.05-0.3 mg/kg ip). Locomotor activity was then assessed in a photocell apparatus for 5 hrs. Acute administration of MOR produced a biphasic locomotor response, including an initial suppression of activity, followed by a delayed facilitation. Chronic administration of MOR led to a decrease in the initial depression (tolerance) and an increase in the delayed facilitation (sensitization). Acute administration of MK-801 prior to MOR produced an effect that mimicked chronic administration of MOR, including a decrease in the initial depression and an increase in the delayed facilitation. Similar effects were seen with other NMDA receptor antagonists, including phencyclidine and memantine. These results, together with others, suggest that NMDA receptor hypofunction may be responsible, at least in part, for tolerance and sensitization to the locomotor effects of opiates. [Supported by NIGMS (GM59833) and NIDA (DA11803)].

Wed29

MATERNAL OPIATE EXPOSURE: LONG-TERM CNS CONSEQUENCES IN THE STRESS SYSTEM OF OFFSPRING.

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Our laboratory investigates the consequences of prenatal exposure to morphine (gestation days 11-18) for adult neurochemistry and behavior. The behavioral results show long-term, sex-dependent alterations in several behaviors including adult sexual behavior. Prenatal morphine exposure inhibits lordosis (reproductive) behavior in young adult female rats. In contrast, morphine-exposed male rats display somewhat enhanced copulatory activities, including more frequent mounting behaviors and significantly shorter post-ejaculatory intromission latencies. The neurochemical results demonstrate that prenatal morphine exposure also has long-term, sex-specific effects on the CNS opioid, catecholamine, excitatory amino acid and GABA systems of exposed progeny. Interestingly, some of our data suggest that neural systems mediating stress responses are affected by prenatal morphine exposure. In male rats, prenatal morphine exposure increases norepinephrine (NE) content and turnover in the hypothalamus, and increases tyrosine hydroxylase-immunoreactivity in the locus coeruleus (LC) and in the paraventricular nucleus of the hypothalamus (PVN). In female rats, these indices are all decreased. NE neurons in the LC mediate the neural responses to stress, and corticotropin-releasing hormone neurons in the PVN are the central neuroendocrine regulators of the hypothalamic-pituitary-adrenocortical axis. Thus, it is possible that many of the diverse, sex-specific effects of prenatal exposure to morphine on adult brain and behavior may have a common denominator: alterations in the function of brain systems that mediate endocrine and neural responses to stress. Supported by: DA05833 to I.V.

Wed30

ADENOVIRAL-MEDIATED EXPRESSION OF THE WILD-TYPE MU OPIOID RECEPTOR, THE T394A MUTANT AND THE μ/δ RECEPTOR IN NEURONS.

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Mutations within the carboxy-terminus of the mu opioid receptor (μOR) have shown this region to be important in desensitization and internalization of this receptor. Two of these mutations, the T394A and the μ/δ receptor, show more rapid internalization and different desensitization profiles than the wild-type (wt) μOR in cell lines. This study describes the use of adenoviral-mediated gene transfer of these different μOR's in cultured dorsal root ganglia (DRG) neurons. The cDNA encoding either the wt μOR, the T394A or the μ/δ (all N-terminus tagged) was placed behind the cytomegalovirus (CMV promoter) at the E1 region of the now replication-defective adenovirus. In addition to the μOR a second CMV promoter was included resulting in the simultaneous expression of green fluorescent protein (GFP). A fourth adenovirus contained only the CMV-GFP cassette. Dissociated DRG cultures from early postnatal mu knockout mice were infected with one of these four adenoviruses and studied after 48 hours. Co-localization of GFP with the tagged μOR receptor within the same cell was confirmed by immuno-histochemistry. Expression of the wt μOR restored DAMGO-induced inhibition of the calcium current (46$\pm$4%, n=6), an effect not observed in those neurons expressing only GFP or un-infected neurons. Similar DAMGO-induced inhibition of the calcium current was seen in those neurons expressing the μ/δ receptor (45$\pm$3%, n=5) but in those neurons expressing the T394A μOR receptor DAMGO-induced inhibition of the calcium current was reduced (13$\pm$4%, n=10).

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DESENSITIZATION OF MU/DELTA-TAIL CHIMERIC RECEPTORS EXPRESSED IN MOUSE DRG NEURONS.

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Studies in non-neuronal or undifferentiated neuronal cell lines have shown that replacing the C-terminal tail of the mu opioid receptor with the C-terminal tail of the delta opioid receptor alters the rate and agonist selectivity of receptor endocytosis. Here we examined the function and desensitization property of this chimeric receptor when expressed in mouse dorsal root ganglion (DRG) neurons in culture. DRG neurons were taken from mu receptor knockout mice, and were infected with viral vectors carrying green fluorescent protein (GFP) and cloned wild type μ opioid receptors or the chimeric receptors. Voltage-activated calcium channel currents were recorded under whole-cell voltage-clamp conditions from infected DRG neurons. Brief exposure of the cells expressing the chimeric receptors to the mu agonist DAMGO or morphine (1 μM, 3 min) reduced Ca^{2+} currents by 45$\pm$3% (n=5) and 72$\pm$4% (n=7), respectively, which was indistinguishable from the effect on neurons expressing wild type mu receptors. Upon prolonged incubation with morphine or DAMGO (1-24 hr), the chimeric receptor developed desensitization to the inhibitory action of mu agonists. Experiments are currently ongoing to compare the time course and extent of functional desensitization of the chimeric receptors with that of wild type mu receptors.

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OPERANT DISCRIMINATIVE STIMULI, NOT CLASSICALLY CONDITIONED STIMULI, REINSTATES FOOD-SEEKING

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Many drug abuse researchers point to the motivational power of drug-associated cues as the fundamental difference between addicts and the rest of the population. Previous studies have had limited success eliciting reinstatement with classically conditioned stimuli (lever press→reward+CS). Instrumentally conditioned, discriminative stimuli (DS→lever press→reward) may offer a better model for human cue-induced relapse. Food-restricted rats were randomly assigned to one of two training conditions. CS_f animals were trained to lever press on a variable interval (VI) 2 min. schedule for food reinforcement (Noyes, 45 mg pellets) and a 20 sec. CS presentation. DS_f rats were yoked to CS_f mates such that each reinforced response made by a CS_f animal triggered a 20 sec. DS presentation in the chamber of the DS_f mate. A lever press during the DS-on period delivered food and a 20 sec. CS. After 7 training sessions, subjects underwent 7 extinction sessions in which neither food nor cues were presented. Finally, reinstatement was measured under extinction-like conditions by counting responses in the 30 min. after one of the following events: 1) free food, 2) DS, 3) CS, 4) a familiar, non-predictive, cue (S_o) presented randomly on a VI 2 min. schedule during training, or 5) no stimulus.

In CS_f animals, only free food elicited more responding than control stimuli (n=9, $p < 0.01$ Tukey post-hoc paired comparisons). On the other hand, both free food and DSs reinstated food-seeking in the DS_f group (n=12, $p<0.05$). CS presentations did not reinstate food-seeking in either group. These data suggest that operant DSs are more powerful motivators of food-seeking than classically conditioned CSs. Ongoing experiments hope to determine whether the same holds true for animals trained to self-administer drugs of abuse.

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EFFECT OF SP_{1-7} ON THE DOPAMINE D2 RECEPTOR mRNA IN MORPHINE DEPENDENT RAT

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The substance P (SP) metabolite SP_{1-7} attenuates the opioid withdrawal syndrome and is also shown to affect the dopamine system. In this study, we investigated the effect of SP_{1-7} on the expression of the D2 receptor transcript and on the withdrawal reaction in morphine dependent rats. Male Spraque-Dawley rats were randomly distributed into two groups. Guide cannula was implanted and aimed at the lateral ventricle. After surgery, the rats were made morphine dependent by twice daily injections of morphine (10mg/kg, s.c.) for 7 days. One group of rats received an injection of SP_{1-7} (25µg / rat) through the cannula half hour before a naloxone challenge. The other group received saline injection and served as control. The naloxone precipitated morphine withdrawal reactions were observed for half an hour. The animals were decapitated 4h after injection. We found that SP_{1-7} decreased the D2 receptor mRNA transcription in nucleus accumbens and frontal cortex. In the behavioral study it was shown that SP_{1-7} attenuated several of the somatic withdrawal symptoms. The decreased D2 receptor mRNA transcription probably reflect an increased dopamine activity in these areas, which, in turn, counteracts the withdrawal reactions.

Wed34

DISTRIBUTION OF ORPHANIN FQ/NOCISTATIN TRANSCTIPTS IN HUMAN IMMUNE CELLS.

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Several studies to date have examined the expression of the ORL-1 receptor in human peripheral blood lymphocytes (PBL) or cell lines. Previously, we had shown that transcripts encoding Orphanin FQ or Nociceptin (OFQ/N), the endogenous ligand to the ORL-1 receptor, were expressed in PHA activated PBL (Arjomand et al (1997) INRC abstract). The aim of these studies was to determine which subset(s) of cell comprising the PBL express OFQ/N. In addition, the cells were also examined for expression of ORL-1. Human peripheral mononuclear cells were sorted using immunomagnetic beads or differentiated in vitro with appropriate growth factors. Total RNA was isolated from the following cells: T helper cells (CD3+, CD4+), Cytotoxic T cells (CD3+, CD8+), B cells (CD19+), Natural Killer cells (CD56+), MCSF differentiated Macrophages, and GMCSF and IL-4 differentiated Dendritic cells. RT-PCR was subsequently used to amplify fragments corresponding to OFQ/N, ORL-1, or β-Actin. All cell types expressed transcripts for the ORL-1 receptor, while CD19+ B cells were observed to express OFQ/N transcripts. Minor expression was also noted in other cell fractions. (This work was supported by a research training program in psychoneuroimmunology 5-T32-MH-19925 and the Hatos Foundation).

Wed35

PAIN-INHIBITORY ROLE OF N/OFQ IN THE SPINAL CORD

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It is known that nociceptin/orphanin FQ (N/OFQ) has both nociceptive and antinociceptive actions. However, it remains to be determined which action is physiologically more important. We have published results that N/OFQ has biphasic actions depending on doses in the nociceptors and spinal synapses, and has postsynaptic antinociceptive actions in spinal cord, by modulating substance P (SP) signaling (PNAS, 95, 10949-10953, 1998; JPET, 291, 308-313, 1999). Mice lacking the gene for the N/OFQ receptor ($NOR^{-/-}$ mice) showed over 1000-fold more sensitive nociception to bradykinin or SP (i.pl.) through an activation of primary afferent SP neuron in the spinal cord. On the other hand, the PGI_2 agonist (i.pl.) was found to induce peripheral nociception through an activation of primary afferent glutamate neuron in the spinal cord, but there was no significant supersensitization in such K/O mice. Although the central nociception induced by SP (i.t.) was also markedly enhanced in $NOR^{-/-}$ mice, there was no significant change in the NK1-receptor expression in the dorsal horn of spinal cord. These findings suggest that nociceptin plays a role as a neurotransmitter of inhibitory recurrent neuron downstream to SP-receptive neurons.

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CONSTRUCTION AND CHARACTERIZATION OF CONSTITUTIVELY ACTIVE MUTANTS OF THE HUMAN OPIOID RECEPTOR-LIKE (ORL_1) RECEPTOR

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Nociceptin, the endogenous ligand of ORL_1 receptor is endowed with supraspinal pronociceptive /anti-opioid activity and modulates peripheral pain perception. Antagonists of the ORL_1 receptor are therefore predicted to suppress pain perception and may act as novel analgesics with low propensity for tolerance. To facilitate the screening of ORL_1 receptor antagonist, a novel approach is being used. We have successfully created the first constitutively active human ORL_1 receptor. Asn^{133} located in the third transmembrane domain was mutated to Trytophan and this mutation was able to turn ORL_1 receptor into constitutively active state. This N133W mutant receptor shows a 3-fold increase over the wild type ORL_1 receptor in basal signaling through coupling to G_{16}. Amongst the various effectors regulated by ORL_1 receptor, stimulation of phospholipase C through coupling to G_{16} holds the best hope for linking receptor activation to high-throughput screening. This increase in basal signaling was shown to be dependent on the amount of DNA being transfected into COS-7 cells. A stable cell line expressing this N133W mutant was constructed in HEK293 cells. Nociceptin was found to have the same EC_{50} and IC_{50} value in stimulating phospholipase C through coupling to G_{16} and inhibiting forskolin-stimulated cAMP accumulation respectively. [Phe1psi (CH2-NH) Gly2]-nociceptin (1-13)-NH2, a proposed antagonist for the ORL_1 receptor behaves as a low potency (IC_{50}=10nM), full agonist in the system and the IC_{50} value was the same between the wild type and the mutant receptor. To further characterize the effect of this mutation on the receptor signaling, ERK activation was investigated. Result shows that the N133W mutant shows a much weaker activation of ERK when compared to the wild type receptor. This might be due to the negative feedback mechanism operating in the cell. These results show that the N133W receptor is constitutively active and might be a useful tool to screen for antagonist with inverse agonistic property.

Wed37

REGULATION OF ATRIAL NATRIURETIC PEPTIDE BY OPIOIDS IN RATS

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The present study was designed to elucidate the opioid recetors involved in regulation of release of atrial natriuretic peptide (ANP) in the rat. We examined the effect of intravenously injected DAMGO, DPDPE, U69,593 and nociceptin by measuring immunoreactive (IR) ANP in plasma of the anesthesized rats. DAMGO (1 mg/kg, i.v.), a μ-agonist, caused a marked increase in plasma IR-ANP (122.9 ± 4.8 pg/ml vs. 288.5 ± 17.5 pg/ml). Treatments with naloxone (Nal; 1 mg/kg, i.v.) or methylnaloxone (met-Nal; 5 mg/kg, i.v.) 20 min prior to DAMGO attenuated the effect of DAMGO by about 40%. DPDPE (1 mg/kg, i.v.), a δ-agonist, increased IR-ANP though less effective than DAMGO (199.2 ± 27.1 pg/ml). The effect of DAMGO on plasma IR-ANP was antagonized by pretreatments of Nal and met-Nal. A κ opioid agonist, U69,593, caused increase of IR-ANP with similar extent with DPDPE. Nal and met-Nal inhibited the effect of U69,593 by about 30%. Nociceptin (1 – 30 nmole/kg) increased plasma IR-ANP dose dependently. Phe-nociceptin also caused increase of plasma IR-ANP but with lesser potency than nociceptin. Effect of nociceptin on plasma IR-ANP was not affected by Nal or met-Nal but by naloxone benzoylhydrazone (1 mg/kg, s.c.). Nal, met-Nal or naloxone benzoylhydrazone did not affect plasma IR-ANP per se.. These results suggest three major types of opioid receptors and nociceptin receptors which are located in peripheral sites are involved in regulation of plasma IR-ANP concentration. (Supported by Korea Research Foundation, 1996)

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ORPHANIN FQ/NOCICEPTIN ATTENUATES MORPHINE TOLERANCE IN RATS USING THE HOT PLATE TEST.

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Recent evidence indicates that the opioid receptor-like (ORL-1) receptor may be involved in morphine tolerance. In the present study we sought to investigate if administration of orphanin FQ (OFQ, also known as nociceptin) would modulate the development of morphine tolerance. Adult male Sprague Dawley rats were treated daily with saline or morphine (10 mg/kg) followed, 15 min later, by an intracerebroventricular injection of artificial cerebrospinal fluid (aCSF) or OFQ (7.5 nmol). Rats then received an additional injection of aCSF or OFQ 1 hr later. Rats received the same treatment for 3 days, except the dose of OFQ was doubled on each day. On day 4, rats were tested on the hot plate apparatus 30 min prior to and 30, 60 and 90 min after morphine. Chronic treatment with OFQ attenuated morphine tolerance without affecting basal nociceptive responses or the antinociceptive effect of morphine in rats. (KL was supported by a KO1 award DA00411 from NIDA and a NARSAD Young Investigator Award)

Wed39

OFQ-INDUCED MODULATION OF LHPA AXIS ACTIVITY: A NEUROANATOMICAL STUDY

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We recently reported that intracerebroventricular administration of the endogenous heptadecapeptide neurotransmitter orphanin FQ (OFQ, also known as nociceptin) produces elevated activity of the limbic hypothalamic-pituitary-adrenal (LHPA) axis. We are currently evaluating the neuroanatomical basis of these effects. In light of the abundance of OFQ and its endogenous receptor in limbic structures, we administered OFQ directly into limbic structures. Plasma was collected 30 minutes after the injections, and corticosterone (CORT) concentrations were subsequently assayed by radioimmunoassay. Injections of OFQ into the central amygdala (CeA), bed nucleus of stria terminalis (BNST), hippocampus, and nucleus accumbens (NAcc) each produced elevated plasma CORT concentrations. Injections into medial prefrontal cortex (MPFC) and ventral tegmental area (VTA) did not affect plasma CORT concentrations. As OFQ exerts a modulatory role on LHPA axis activity in some, but not all limbic structures, it appears that these actions may be specific to particular aspects of LHPA axis function. We are continuing to evaluate the neuroanatomical substrates that mediate the OFQ-induced modulation of LHPA axis activity, and we are investigating the neurochemical basis of these alterations.

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DIRECT IDENTIFICATION OF A PEPTIDE BINDING DOMAIN IN THE ORL1 RECEPTOR WITH [Bpa^{10}, ^{125}I-Tyr^{14}]-NOCICEPTIN.

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A photo-affinity labelling approach has been implemented in order to further our understanding of the interaction of nociceptin with the ORL1 receptor, using the iodinated nociceptin derivative, [*p*-benzoyl-L-Phe^{10}, ^{125}I-Tyr^{14}]-nociceptin ([Bpa^{10}, ^{125}I-Tyr^{14}]noc). In recombinant CHO cells expressing the human ORL1 receptor, [Bpa^{10},Tyr^{14}]noc binds the receptor with high affinity (K_i ~ 0.7 nM), and is as potent as nociceptin (EC_{50} ~ 0.5 nM) in the inhibition of forskolin-induced cAMP synthesis. UV irradiation of the reversible complex formed by the hORL1 receptor and [Bpa^{10}, ^{125}I-Tyr^{14}]noc yields a macromolecular species of M_r ~ 65 kDa, as estimated by SDS-PAGE. Digestion of the radio-labelled 65 kDa species with tissue kallikrein (-Leu/Phe-Arg|Xaa-), or endoproteinase Glu-C (-Glu|Xaa-), generates radioactive fragments of Mr ~ 6.5 and ~ 5.5 kDa, respectively. Treatment of the kallikrein digestion product with endoproteinase Glu-C yields a single radioactive fragment of Mr ~ 3.5 kDa. Based upon the theoretical proteolytic fingerprint of the hORL1 receptor, these results identify the [Bpa^{10}, ^{125}I-Tyr^{14}]noc-reactive region as hORL1-[296-302] - Thr-Ala-Val-Ala-Ile-Leu-Arg - comprising the C-terminus of extracellular loop 3, and the N-terminus of transmembrane helix VII. Molecular modelling of the hORL1 receptor complex with [Bpa^{10}]noc-[1-13]-NH_2, on the basis of our earlier model of the hORL1 receptor complex with nociceptin [Topham, C.M. *et al*. (1998) *Prot. Eng.* 11, 1163-1179], predicts the reactive ketone oxygen of Bpa to lie in close proximity to the sidechain of Ile^{300}.

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COCAINE SENSITIZATION INCREASES THE LEVEL OF ORPHANIN FQ-IMMUNOREACTIVITY IN THE RAT HYPOTHALAMUS.

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The present study was designed to investigate if chronic intermittent cocaine treatment would result in changes in the level of orphanin FQ/nociceptin-immunoreactivity (OFQ-IR) in certain brain areas in rats. Adult male S/D rats were treated with saline or cocaine (40 mg/kg) once daily for 3 days and then sacrificed on day 11. Different brain regions were dissected out, placed in ice-cold acid-acetone, sonicated and spun down. A 1:10 dilution of the supernatant was assayed in quadriplicates using a sensitive solid phase radioimmunoassay developed in our laboratory. Cocaine treatment resulted in an increase in the level of OFQ-IR in the hypothalamus. Other brain regions tested included the medulla, pons, midbrain, thalamus, hippocamus, striatum and cortex. OFQ-IR levels did not change in these regions. This is the first indication that the OFQ system may play a functional role in cocaine sensitization. (Supported in part by a KO1 award DA00411 from NIDA and a NARSAD Young Investigator Award to KL)

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ORPHANIN FQ (OFQ)/NOCICEPTIN (N) AND THE C-TERMINAL PEPTIDE OF PREPRO OFQ/N ARE INTERNALIZED BY AN AMYGDALA-DERIVED CELL LINE (AR5) AND SH5Y CELLS; OFQ/NOCICEPTIN (OFQ/N) INTERNALIZATION IS MODULATED BY μ AND δ OPIOIDS.

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We wanted to determine whether neuropeptides derived from the recently discovered prohormone ppOFQ/nociceptin were internalized by cells expressing their receptors. SH-SY5Y cells were incubated (30 min) with ^{125}I-OFQ/N, in the presence and absence of excess cold OFQ/N or the opioid ligands DPLPE (δ) and DAMGO (μ). Neither DAMGO nor DPLPE alone was capable of significant inhibition of OFQ uptake. However, when incubated together, OFQ/N uptake was inhibited by 50%. In order to determine the internalization domain of OFQ/N we incubated SH-SY5Y cells with ^{125}IOFQ/N and excess cold OFQ/N1-7, 1-11, and 1-17. All three peptides abolished uptake, indicating either the internalization domain of OFQ/N is in the N-terminus or partial agonist activity inhibits the uptake process. In parallel experiments AR5 cells were incubated with either ^{125}IOFQ/N or ^{125}IppOFQ/N160-187 in the presence and absence of cold ligand. Both peptides were taken up robustly by these cells indicating that this amygdalar cell line expresses two receptors for peptides derived from the OFQ/N precursor. Taken together these data suggest that uptake of peptide ligands from the OFQ/N family is involved in cell signaling and is regulated by opioids. Supported by NIH DA11282(RGA)

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THE EFFECT OF MATERNAL SEPARATION ON TISSUE LEVELS OF NOCICEPTIN IN RAT BRAIN.

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Maternal separation during the critical period of development, i.e. the first 21 days of life in rats, enhances the neuroendocrine responses to stress both at the behavioural and the neurochemical levels in adult life. The aim of this study was to investigate if three hours of maternal separation can induce long-term neurochemical changes in brain nociceptin/orphanin FQ immunoreactive (ir) levels in male Sragues-Dawley rats. 12 rat pups were removed from their home cages and mothers and placed in individual containers for three hours daily for the first 21 days of life. 12 rats served as controls and were left undisturbed with their mothers. In order to investigate long-term effects of maternal separation the rats were then left undisturbed for one month. After this period the rats were decapitated and nociceptin ir-levels were determined in several brain areas. Maternally separated rats had significantly decreased ir-levels of nociceptin in the hypothalamus, striatum, hippocampus and the periaqueductal gray as compared to controls. The results indicate that manipulations early in life can induce persistent neurochemical changes in the nociceptin system.

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EVIDENCE FOR CROSS TALK BETWEEN ORL-1 AND μ OPIOID RECEPTORS IN HUMAN NEUROBLASTOMA CELLS.

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Previously we reported that OFQ/N pretreatment (100 pM, 1 hr) reduced the ability of morphine to inhibit cAMP accumulation in intact BE(2)-C cells **(**Altememi GF and Standifer KM, 1999, *The Pharmacologist* **41**:124**).** We now report that DAMGO pretreatment (1 μM, 1 hr) reduces OFQ/N-mediated inhibition of cAMP accumulation in intact BE(2)-C cells by 65%. To rule out the possible role of heterologous desensitization in DAMGO's effect on OFQ/N activity and to further investigate a potential μ/ORL-1 receptor interaction, BE(2)-C cells were pretreated with the irreversible μ antagonist, β-FNA (1 μM, 1 hr). As expected, β-FNA blocks DAMGO-mediated inhibition of cAMP and μ receptor binding more than 85%; unexpectedly, it decreases the OFQ/N-mediated cAMP response 96% ($p<0.05$). Concentrations of β-FNA up to 10 μM fail to compete for more than 16% of specific [^{3}H]OFQ/N binding in membranes from either BE(2)-C or CHO-KOR3 cells, indicating that the effects of β-FNA aren't mediated through the OFQ/N binding site on ORL-1. In parallel studies, cells were pretreated with antisense DNA (1 μM, 5 d) directed against TM5 of hORL-1 to determine the effect of ORL-1 "blockade" on μ receptor activity. Antisense DNA treatment reduces OFQ/N-mediated inhibition of cAMP accumulation 85% ($p<0.05$), with no effect on the μ response of DAMGO. Thus, an interesting interaction or cross talk between ORL-1 and μ opioid receptors exists in BE(2)-C human neuroblastoma cells, and further studies to delineate this interaction are ongoing. This work was supported by NIDA (DA10738)

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ELECTROACUPUNCTURE ANALGESIA IN ORPHANIN FQ (NOCICEPTIN) AND OPIOID RECEPTOR-LIKE RECEPTOR (ORL_1) KNOCKOUT MICE *

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OFQ has been shown to modulate opioid analgesia, nociception, and electroacupuncture (EA) analgesia (EAA) in rats. It has also been demonstrated that EA activates endogenous opioid peptides and other anti-opioid peptides as well. In the present study, we aim to investigate the role of OFQ and ORL_1 in EAA with knockout mice. Two pairs of acupuncture needles were inserted into acupoints ST 36 and SP 6, the needles were connected to one of the output channels of an electric stimulator HANS through electric wires. EA parameters were as follows: constant current, square wave pulses, 1.0-1.2-1.4 mA in intensity increased with stepwise fashion, 10 min for each intensity, 100 Hz or 2 Hz in frequency with 0.2 ms or 0.6 ms as pulse width respectively. Tail flick latencies (TFL) evoked by radiant heat were measured before, during and after EA stimulation. The average of the 3 TFL values before EA was taken as basal TFL. OFQ or ORL_1 mice of wild type or homozygous mutation were divided randomly into 3 groups: needle control (needles only without electric stimulation), EA 100 Hz or 2 Hz. The net change of the peak value among the 6 time points during and after EA relative to its own basal TFL was calculated for each mouse, and then analyzed by Analysis of Variance (ANOVA). The results so far indicate that EA at both 100 Hz and 2Hz had tendencies to provide stronger analgesia in both OFQ and ORL_1 knockout mice than in wild type mice. It is speculated that OFQ and ORL_1 system antagonizes acupuncture analgesia *in vivo*. (* Supported by NIDA/ INVEST grant and DA-09040, NIH, USA).

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CHARACTERIZATION OF THE HUMAN PREPRO-NOCICEPTIN GENE AND ITS REGULATION BY cAMP

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We have sequenced and cloned 1.1 kb of the 5'-untranslated region of the human prepronociceptin (ppNOC) gene into a luciferase reporter plasmid, pGL3 Basic Vector. By primer extension analysis, we have determined that the start site of transcription lies within the human ppNOC cDNA sequence published by Mollereau and colleagues. There are 2 cyclic AMP (cAMP) response elements with the consensus sequence CGTCA and a pyrimidine rich initiator (Inr) motif with the sequence CACTCCTC within 250 bp of the start of transcription. We designed various constructs around these sites, performed transient transfections and measured luciferase activity in NS20Y cells. We found that the Inr sequence does not inhibit transcription of the ppNOC promoter as it does for the preprodynorphin gene. Upon stimulation with forskolin + IBMX, there is only a modest cAMP response even when both cAMP sites are present. Mollereau and colleagues have shown that there is an intron 23 bp ahead of the ATG translation start codon in the ppNOC gene. When we designed plasmid constructs containing promoter region up to the intron and compared it to constructs lacking a 120 bp piece adjacent to the intron (on the 5' side), we found a significant increase in transcriptional activity. We are currently investigating what enhancer element(s) present in this region might be responsible for differences in transcriptional activation and regulation of the human ppNOC gene.

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AN *O*-PHTHALALDEHYDE DERIVATIVE OF NALTRINDOLE AS A FLUOROGENIC AFFINITY LABEL

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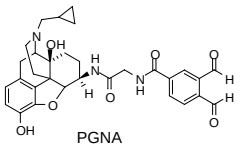
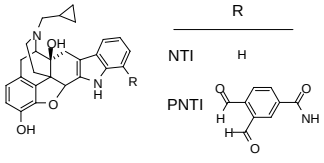
The design of the first fluorogenic opioid receptor affinity label PGNA has been reported recently. The design rationale of this compound was based on the formation of a fluorescent isoindole when *o*-phthalaldehyde (OPA) reacts with primary amines in the presence of thiols. The receptor-based residues that are covalently bound to PGNA are believed to be a lysine and cysteine. In an attempt to develop a δ-selective fluorogenic affinity label based on the same approach we have synthesized an OPA derivative (PNTI) of naltrindole (NTI). Preliminary experiments conducted on the mouse vas deferens preparation demonstrated that PNTI is an extremely potent δ-agonist (IC_{50}= 0.12 nM). Furthermore, the agonism is wash-resistant suggesting covalent association with δ-opioid receptors. The detailed pharmacological characterization of PGNA and PNTI and the significance of these results will be presented.





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ENDOMORPHIN - SUBSTANCE P AGONIST CHIMERAS

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It is commonly accepted that substance P and opioid peptides play opposite role in pain signal transmission in spinal cord. Nevertheless, low concentration of exogenous substance P accelerates analgesic activity of opioids and delays development of tolerance. This observation was a base for search of opioid peptide - substance P chimeras with optimal proportion of opioid and substance P agonist activites. In this communication, the biological properties of novel peptide (ESP7) in which endomorphin and substance P C-terminal fragment have been hybridized. Resulted peptide expressed "naloxon-reversible" analgesic activity without induction of tolerance.

Tyr-Pro-Phe-Phe-Gly-Leu-MetNH_2
endomorphin******
**********substance P

Fig. Amino acid sequence of ESP7

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BIOLOGICAL PROPERTIES OF N-GUANIDYNO OPIOID PEPTIDES

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Transformation of amino group of opioid peptides into guanidino group, resulted in shifting affinity to μ-receptor types. Therefore, such modification was not attractive in searching for δ- or κ-opioid receptor selective ligands. In our recent avenue of searching for site directed analgesics, we have predicted that multireceptor opioid peptide ligands could be clinically attractive analgesics. In this case modification of enzymatic resistance or biological barrier permeabilities of ligands became more important issue than selectivity. From this respect, guanidinations of opioid peptides is very promising approach. In this communication, pharmacological properties of several N-guanidino-opioid peptides will be presented. The comparison of pharmacological properties of guanidino-peptides with their N-amino parent compounds evidence their higher biological enzymatic resistance as well as biological barrier permeabilities.

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BINDING AFFINITY AT HUMAN CLONED OPIOID RECEPTORS FOR A SERIES OF 3,4-DIMETHYL-4-(3-HYDROXYPHENYL)PIPERIDINES

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We have previously reported potent pure opioid antagonist activity for structures derived from the 3,4-dimethyl-4-(3-hydroxyphenyl)piperidine core platform. Characterization of receptor binding affinity for 16 analogs from the series was carried out using [^{3}H]-diprenorphine at human mu-opioid (h-MOR) and kappa-opioid (h-KOR) receptors expressed in cloned cell lines, and with [^{3}H]-naltrindole at human delta-opioid receptors (h-DOR). At h-MOR receptors, the highest affinity compound was LY255582 (J. Med. Chem. 1993, 36, 2842-2850), Ki = 0.017 nM. In comparison, naltrexone had 28 fold weaker affinity for h-MOR receptors, with Ki = 0.47 nM. At h-KOR, LY255582 had Ki = 0.63 nM. Naltrexone had 4 fold weaker affinity for h-KOR receptors, with Ki = 2.5 nM. At h-DOR, LY255582 had Ki = 4.2 nM. In contrast, naltrexone was 8 fold weaker in affinity for h-DOR, with Ki = 33 nM. In terms of relative selectivity, LY255582 showed a h-KOR to h-MOR ratio of 37, while naltrexone had a h-KOR to h-MOR ratio of 4. Similarly, LY255582 showed a h-DOR to h-MOR ratio of 247, while naltrexone had a h-DOR to h-MOR ratio of 70.

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R-ATC-ILE^{5,6} DELTORPHIN II HAS PARTIAL AGONIST ACTIVITY AT THE MOUSE DELTA OPIOID RECEPTOR.

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R-Atc-Ile^{5,6} Deltorphin II (RATGLU) is a selective delta opioid receptor agonist, with selectivity for mu/delta being >20000 and an IC_{50} of 0.09nM in the mouse vas deferens. However, we have previously reported differences between the response of RATGLU and deltorphin I to a GTP analogue (GMPPNP) in rat brain homogenates. In this study we have used quantitative autoradiography, homogenate binding and the mouse vas deferens bioassay to further characterise the behaviour of this ligand at the mouse delta-opioid receptor. In both homogenate binding and autoradiography, [^{3}H]RATGLU was insensitive to GMPPNP. In addition, in brain membranes [^{3}H]RATGLU recognised a significantly greater number of receptors compared to deltorphin I. This suggested that [^{3}H]RATGLU may not behave as a full agonist at the brain delta opioid receptor. In the mouse field-stimulated vas deferens RATGLU caused complete inhibition of twitch response. This inhibition was reversible by naltrindole, a selective delta-opioid antagonist. However, preincubation of the vas deferens with RATGLU (IC_{10-20}) was shown to antagonise the response to deltorphin I. From these findings we conclude that despite the full inhibition of twitch height in the mouse vas deferens, RATGLU might behave as a partial agonist at both the peripheral and central mouse delta opioid receptor.

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SUPER-DALDA: A POLAR, HIGHLY POTENT AND SELECTIVE μ OPIOID AGONIST

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The tetrapeptide DALDA (H-Tyr-D-Arg-Phe-Lys-NH_2) is a polar and selective μ opioid agonist showing poor penet-ration of the placental and blood-brain barriers. In an effort to enhance the potency of DALDA, analogues containing 2',6'-dimethyltyrosine (Dmt), N,2',6'-trimethyltyrosine (Tmt), 2'-methyltyrosine (Mmt) or 2'-hydroxy,6'-methyltyrosine (Hmt) in place of Tyr^1, or ornithine (Orn) or α,γ-diaminobutyric acid (A_2bu) in place of Lys^4, were synthesized. All compounds displayed high naloxone-reversible μ agonist potency in the guinea pig ileum (GPI assay), and high μ receptor binding affinities and high μ vs. δ and μ vs. κ receptor selectivities in the rat and guinea pig brain membrane binding assays. The most potent compound was [Dmt^1]DALDA (SUPER-DALDA) with an IC_{50} of 1.41 nM in the GPI assay and with K_i^{μ}, K_i^{δ} and K_i^{κ} receptor binding affinity constants of 0.143, 1240 and 26.3 nM, respectively. Determination of the capacity factor of this compound by HPLC confirmed its polar character. Because of its extraordinary μ agonist potency, high μ selectivity, high polarity (charge of 3+) and metabolic stability, [Dmt^1]DALDA has potential for use in obstetrical and peripheral analgesia.

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HS 378 – A NEW DELTA-SELECTIVE AND IMMUNOSUPRESSIVE OPIOID ANTAGONIST

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Several drugs or strategies have been employed in an effort to shut down the cascade of the inflammatory events taking place in rheumatoid arthritis. It has been reported that delta-selective opioid antagonists exhibit immunosuppressive properties. HS 378 is a new opioid antagonist with very high affinity and selectivity for the delta-opioid receptor in brain as indicated by receptor binding assays. This compound was found to be a potent inhibitor of concanavalin A-induced proliferation of T-lymphocytes at concentrations of 10^{-5}-10^{-6} M. In order to assess the immunosuppressive effect in vivo, HS 378 (0.5 - 8 mg/kg/day, i.p.) was tested in adjuvant arthritic rats. Serum levels of cartilage oligomeric protein (COMP) were measured by immunoassay. Opioid-treated animals showed lower level of this marker (11-24%) compared to arthritic non-treated rats. The present results suggest that HS 378 has an important immunomodulator role in the pathogenesis of adjuvant arthritis acting by regulating the cellular immune response. Thus, it may be a promising therapeutic compound for human inflammatory arthritic diseases.

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IN VIVO ROLE OF PREPRO-OFQ/N160-187 IN PAIN REGULATION

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Recently discovered orphanin FQ/nociceptin (OFQ/N) neuropeptide, like all known opioid peptides is encoded in a prohormone. Although there exists another potential peptide C-terminal to OFQ/N, prepro-(pp)OFQ/N160-187, little is known of its pharmacological actions and physiological roles. Here we studied pharmacological actions and its *in vivo* signaling of ppOFQ/N160-187 as well as its physiological role in pain regulation in mice. In peripheral nociception test measuring flexor responses, intraplantar injection (i.pl.) of this peptide at doses between 1 fmol and 1 pmol elicited a dose-dependent action. Unlike in the case with OFQ/N, the action was not affected by intraplantar injection of pertussis toxin, phospholipase C inhibitor nor NK1 antagonist, which had been given through another cannulae adjacent to that for ppOFQ/N160-187. Instead this action was blocked by cholera toxin. The nociception by ppOFQ/N160-187 (i.pl.) was blocked by intrathecal injection of NMDA antagonist, but not by NK1 antagonist nor AMPA/kainate antagonist. The intrathecal injection of 3 micro g of anti-ppOFQ/N160-187 rabbit IgG significantly increased the latency in the tail-flick test in mice, while the same amount of preimmune rabbit IgG did not affect it. All these findings suggest that ppOFQ/N160-187 exerts nociception through nociceptors, receptor and its downstream mechanisms different from the case with OFQ/N, and that itself may play an *in vivo* stimulatory role in pain regulation.

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AN ENZYMATICALLY STABLE KYOTORPHIN ANALOG INDUCES PAIN IN SUBATTOMOL DOSES

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Since kyotorphin (H-Tyr-Arg-OH) is known to be rapidly degraded by aminopeptidases, enzymatically stable analogs of kyotorphin and its antagonist (H-Leu-Arg-OH) were synthesized and tested in a peripheral nociception assay. Intraplantar injection of the enzymatically stable N-methylated kyotorphin analog Tyr(NMe)-Arg-OH at extremely low doses produced nociceptive flexor responses in a dose-dependent manner in mice. The ND_{50} (dose producing 50% of the maximal reflex in the nociception assay) was 0.55 amol (20,000-fold lower than that of kyotorphin) and a significant response was observed with this compound at a dose of 0.01 amol (6000 molecules!). The stable nociception produced by 10 amol of Tyr(NMe)-Arg-OH was completely blocked by 100 fmol of the kyotorphin antagonist H-Leu-Arg-OH and by 10 amol of its enzymatically stable N-methylated analog [Leu(NMe)-Arg-OH]. The Tyr(NMe)-Arg-OH-induced nociception was also completely blocked by 1 pmol of the neurokinin-1 antagonist CP-99994, but not by 1 pmol of its inactive isomer (CP-100263). These results suggest that the nociceptive effect produced by Tyr(NMe)-Arg-OH in subattomol doses occurs via specific interaction with the kyotorphin receptor and that the extraordinary potency observed may be the result of amplification through local substance P release.

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MODELLING AND SEQUENCE ANALYSIS OF A NOVEL OPIOID RECEPTOR FROM THE ZEBRAFISH PROVIDES NEW INSIGHTS INTO LIGAND-RECEPTOR INTERACTIONS.

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Opioid-like peptide precursors and receptors are thought to be present throughout much of evolution. Recently, genes encoding putative opioid receptors have been isolated and sequenced from several primitive non-mammalian species. For example, ZFOR1 (ZebraFish Opioid Receptor 1) from the teleost *Danio rerio* displays highest sequence identity (66%) and homology (90%) to the mammalian δ opioid receptor. However, it displays a pharmacological profile distinct from those of the mammalian δ, μ or κ receptors. In the current study, a model of ZFOR1 is presented which is based on sequence homology with opioid and other G protein coupled receptors (GPCRs). A binding pocket within the transmembrane domains has been identified, and various opioid ligands have been docked into it. The role of various ZFOR1 residues in its unique pharmacology will be discussed, with reference to the docked ligand alignments and mutagenesis data from the mammalian opioid receptors. Finally, an evolutionary trace analysis that incorporates the sequence of ZFOR1 together with those of a wide range of GPCRs provides insights into the role of various residues in opioid receptor structure, ligand binding and activation.